

ANA–NINDS CAREER DEVELOPMENT SYMPOSIUM

*Advancing the Next Generation of
Academic Neurology Leaders*



OCTOBER 16–17, 2026



SHERATON SAN DIEGO RESORT
SAN DIEGO, CALIFORNIA



MENTORSHIP
& NETWORKING



RESEARCH
ADVANCEMENT



LEADERSHIP
DEVELOPMENT



FUNDING
STRATEGIES



CAREER
ADVANCEMENT

FINAL PROGRAM

PRESENTED BY THE AMERICAN NEUROLOGICAL ASSOCIATION
AND
THE NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE

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ANA-NINDS Career Development Symposium

October 16-17, 2026

Sheraton San Diego Resort
San Diego, CA

SPONSORED BY:

The American Neurological Association

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Dimitri Krainc, MD, PhD, FANA
President
Alexandra Nelson, MD, PhD
Chair



National Institute of Neurological Disorders and Stroke

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Bethesda, MD 20892-2540
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Letitia "Tish" Weigand, PhD
Director
Elizabeth Sypek, PhD
Director



National Institute of
Neurological Disorders
and Stroke

COURSE GOALS

The ANA-NINDS Career Development Symposium is designed to provide you with the essential tools to enhance your ability to write successful grant proposals, to obtain grant funding from NIH and other institutions and build an impactful academic career. This course is held in conjunction with the ANA2026 Annual Meeting.

This symposium, now in its twentieth year, is designed for K08, K12 and K23 recipients and will be chaired by senior neurologists and neuroscientists who have proven success in career building and navigation, scientific grant writing, networking, and balancing clinical and research efforts. In addition, senior staff from NINDS will provide advice concerning the mechanisms involved in grant submission and evaluation.

COURSE EVALUATION

Participants are asked to complete the evaluation by **Friday, November 13, 2026**. We sincerely appreciate your constructive feedback and comments and ask that you please take a few moments to complete the evaluation.



National Institute of
Neurological Disorders
and Stroke



2026 ANA-NINDS Career Development Symposium Agenda

All times are listed in Pacific Daylight Time (PDT)

Silver Pearl Ballroom 1

Friday, October 16, 2026

2:00 PM - 2:30 PM	Registration (Silver Pearl Ballroom 1)
2:30 PM - 2:45 PM	Welcome and Goals for the Meeting <i>Chair: Alexandra Nelson, MD, PhD, University of California, San Francisco</i>
2:45 PM - 3:15 PM	View of NINDS Leadership 2026 <i>Speaker: Amy Adams, PhD, National Institute of Neurological Disorders and Stroke (NINDS)</i>
3:15 PM - 4:00 PM	Setting Yourself up for Success <i>Speakers: Letitia "Tish" Weigand, PhD, National Institutes of Health K Paul Rezaizadeh, PhD, National Institutes of Health</i>
4:00 PM - 4:45 PM	Meet Your Peers and Colleagues Networking Sessions <i>Table Assignments by Research Focus (20 min) and Clinical vs Lab (20 min). Please find your table assignments on the back of your name badge.</i>
4:45 PM - 5:00 PM	ANA Presidential Address <i>Speaker: Dimitri Krainc, MD, PhD, FANA, Northwestern University</i>
5:00 PM - 6:00 PM	K to Independence Panel – How I Did It <i>Moderator: Ethan Goldberg, MD, PhD, FANA, The Children's Hospital of Philadelphia</i> Panelists: <i>Katy Cross, MD, PhD, University of California, Los Angeles Sattar Khoshkoo, MD, Mass General Brigham/Harvard Medical School Joanna Mattis, MD, PhD, University of Michigan Rujuta Wilson, MD, University of California, Los Angeles</i>
6:00 PM - 6:30 PM	Leadership: Lessons from Academia and Life <i>Speaker: Annapurna Poduri, MD, MPH, FANA, Boston Children's Hospital</i>
6:30 PM - 7:15 PM	Opening Buffet Dinner
7:15 PM – 8:45 PM	Chairs View Panel <i>Moderator: Alexandra Nelson, MD, PhD, University of California, San Francisco</i> Panelists: <i>Amy Brooks-Kayal, MD, FANA, UC Davis School of Medicine Jin-Moo Lee, MD, PhD, FANA, Washington University in St. Louis Thabele Leslie-Mazwi, MD, FANA, University of Washington</i>

Saturday, October 17, 2026

7:00 AM - 8:00 AM	Breakfast
7:30 AM – 8:00 AM	Poster Set-Up (Poster Set-up in Silver Pearl Ballroom 2)
8:00 AM - 9:00 AM	Building Your Portfolio and Collaborations Moderator: Sami Barmada, MD, PhD, FANA, University of Michigan Panelists: Aimee Kao, MD, PhD, University of California, San Francisco Anita Koshy, MD, FANA, University of Arizona Timothy Miller, MD, PhD, FANA, Washington University in St. Louis
9:00 AM - 9:15 AM	Coffee Break and Head to Breakout Sessions Please find your breakout assignment on the back of your name badge.
9:15 AM - 11:30 AM	Breakout Session 1 1 st – 2 nd Year Awardees – Abstract for First Major Paper Discussion 3 rd – 5 th Year Awardees – Aims Page for R01 Discussion
11:30 AM - 12:30 PM	Why Be a Clinician-Scientist in 2026? Moderator: Catherine Chu, MMSc, FANA, Johns Hopkins University Panelists: Alice Chen-Plotkin, MD, PhD, FANA, University of Pennsylvania Aaron Boes, MD, PhD, University of Iowa Jaishri Blakeley, MD, Johns Hopkins University Henry Paulson, MD, PhD, FANA, University of Michigan
12:30 PM - 1:15 PM	Lunch
1:15 PM - 2:15 PM	Challenges and Problem Solving Moderator: William Zieger, MD, PhD, University of California, Los Angeles Panelists: Nicole Coufal, MD, PhD, FANA, University of California, San Diego Peyman Golshani, MD, PhD, FANA, University of California, Los Angeles Anli Liu, MD, MA, New York University Langone Health Mercedes Paredes, MD, PhD, FANA, University of California, San Francisco
2:15 PM - 2:30 PM	Coffee Break and Head to Breakout Sessions Please find your breakout assignments on the back of your name badge.
2:30 PM - 4:00 PM	Breakout Session 2 <i>"What Are the Biggest Challenges You Are Facing and How Can We Help Each Other?"</i>
4:00 PM - 5:00 PM	Poster Session

Faculty List

NAME	LAST	CREDENTIALS	INSTITUTION	ROLE
Amy	Adams	PhD	National Institute of Neurological Disorders and Stroke	Speaker
Sami	Barmada	MD, PhD, FANA	University of Michigan	Moderator, Mentor
Jaishri	Blakeley	MD	Johns Hopkins University	Panelist, Mentor
Aaron	Boes	MD, PhD	University of Iowa	Panelist, Mentor
Amy	Brooks-Kayal	MD, FANA	UC Davis School of Medicine	Panelist, Mentor
Alice	Chen-Plotkin	MD, FANA	University of Pennsylvania	Panelist, Mentor
Catherine	Chu	MD	Johns Hopkins University	Moderator, Mentor
Nicole	Coufal	MD, PhD, FANA	University of California, San Diego	Panelist, Mentor
Katy	Cross	MD, PhD	University of California, Los Angeles	Panelist, Mentor
Ethan	Goldberg	MD, PhD, FANA	The Children's Hospital of Philadelphia	Moderator, Mentor
Peyman	Golshani	MD, PhD, FANA	University of California, Los Angeles	Panelist, Mentor
Kristin	Guilliams	MD, FANA	Washington University School of Medicine St. Louis	Mentor
Aimee	Kao	MD, PhD	University of California, San Francisco	Panelist, Mentor
Sattar	Khoshkoo	MD	Mass General Brigham/Harvard Medical School	Panelist, Mentor
Anita	Koshy	MD, FANA	University of Arizona	Panelist, Mentor
Dimitri	Krinc	MD, PhD, FANA	Northwestern University	Speaker
Jin-Moo	Lee	MD, PhD, FANA	Washington University in St. Louis	Panelist, Mentor
Thabele	Leslie-Mazwi	MD, FANA	University of Washington	Panelist, Mentor
Anli	Liu	MD, MA, FANA	New York University Langone Health	Panelist, Mentor
Joanna	Mattis	MD, PhD	University of Michigan	Panelist, Mentor
Timothy	Miller	MD, PhD, FANA	Washington University in St. Louis	Panelist
Alexandra	Nelson	MD, PhD	University of California, San Francisco	Moderator, Chair, Mentor
Mercedes	Paredes	MD, PhD, FANA	University of California, San Francisco	Panelist, Mentor
Henry	Paulson	MD, PhD, FANA	University of Michigan	Panelist, Mentor
Annapurna	Poduri	MD, MPH, FANA	Boston Children's Hospital	Speaker
K Paul	Rezaizadeh	PhD	National Institutes of Health	Speaker

Letitia	Weigand	PhD	National Institutes of Health	Speaker
Rujuta	Wilson	MD	University of California, Los Angeles	Panelist, Mentor
William	Zeiger	MD, PhD	University of California, Los Angeles	Moderator, Mentor



Alexandra Nelson, MD, PhD
University of California, San Francisco

Dr. Alexandra Nelson is the Richard and Shirley Cahill Endowed Chair in Parkinson's Disease Research at UC San Francisco. Dr Nelson received her MD/PhD training at UC San Diego, completed her residency and fellowship training at UCSF, and joined the faculty in 2014. In the lab, her research group investigates the cellular and circuit basis of movement disorders, using electrophysiology, optogenetics, and other optical methods in mouse models of disease. In the clinic, she focuses on the care of patients and families with Huntington's Disease, atypical parkinsonian disorders, and Spinocerebellar Ataxias.



K. Paul Rezaizadeh, PhD
National Institutes of Health

Dr. K. Paul Rezaizadeh is Program Director for NINDS Clinician Career Development Awards and Institutional Training Grants. He earned his PhD at the University of South Florida studying proteolysis in Alzheimer's disease, then joined Cedars-Sinai Medical Center's Regenerative Medicine Institute to develop a novel rat model of Alzheimer's and explore immunotherapies for neurodegenerative disease. At Pennington Biomedical Research Center, he received an F32 to study neural circuits regulating metabolism using chemogenetic and optogenetic tools. Before joining NINDS, he trained in research administration at the University of Maryland managing sponsored research programs for the Institute for Governmental Service and Research.

Elizabeth Sypek, PhD
National Institute of Neurological Disorders and Stroke

Dr. Elizabeth Sypek is a Program Manager for HEAL (The Helping End Addiction Long-term Initiative) and NINDS in the NINDS Office of Training and Workforce Development. She manages HEAL and pain-related training and early career funding opportunities. Prior to joining NINDS, Elizabeth completed her Ph.D. in Neuroscience at Stanford University with Dr. Gregory Scherrer studying microglial transcriptional responses to chronic opioid exposure and neuropathic pain. She then completed a postdoctoral fellowship at Johns Hopkins University further studying neuro-immune interactions in pain and itch.



Letitia "Tish" Weigand, PhD
National Institutes of Health

Dr. Tish Weigand is the Director of the NINDS Office of Training and Workforce Development at NINDS. Dr. Weigand's career in science has hit the trifecta of academia, government, and industry. She began at the lab bench conducting research at the intersection of neuroscience, physiology and immunology, after which she entered government service at NINDS as a program analyst and manager in the Training Office. There she oversaw institutional training programs and many other initiatives for a number of years before moving on to work in the pharmaceutical industry as a Medical Science Liaison for UCB, serving as a regional expert on the science underlying the company's epilepsy portfolio. She recently made her return to NINDS to create and lead programs that support research training and career development for graduate students, postdocs, and early career physician scientists. Dr. Weigand holds a PhD from Johns Hopkins University and completed a postdoctoral training in neuroscience at George Washington University. She is passionate about preparing and equipping the next generation of scientists and professionals for success. Throughout her career she has served as a speaker and mentor throughout the biomedical and neuroscience communities.



Amy Bany Adams, PhD

National Institute of Neurological Disorders and Stroke

Dr. Amy Bany Adams serves as acting director and deputy director of NINDS where she works to advance the mission of the Institute to improve fundamental knowledge about the brain and the nervous system and to use that knowledge to reduce the burden of neurological disorders. She served in multiple senior scientific, operational and communication leadership roles since joining NINDS in 2013, and she has worked at NIH since 2004. Dr.

Adams earned her PhD in cell biology from Yale University School of Medicine, where her research focused on the genetic and cellular basis for neurotransmitter control of behavior in *C. elegans*. She received her BS in biology from the Massachusetts Institute of Technology.



Sami Barmada, MD, PhD, FANA

University of Michigan

Dr. Sami Barmada received his MD and PhD from Washington University in St. Louis and completed his neurology residency and fellowship at the University of California, San Francisco. He moved to the University of Michigan as an assistant professor of neurology in 2013, was promoted to associate professor in 2020, became director of the University of Michigan Brain Bank in 2021 and an associate director of the MSTP in 2024. He earned the ASCI Young Physician Scientist Award in 2014, the ANA Derek Denny Brown Young Neurological Scholar Award in 2022 and the NINDS/NIH Jacob Javits Neuroscience Investigator Award in 2025.



Jaishri Blakely, MD

Johns Hopkins University School of Medicine

Dr. Jaishri Blakely is a clinician-scientist focused on nervous system tumors.

She directs the Johns Hopkins Comprehensive Neurofibromatosis Center and provides care for people with NF1, the schwannomatoses and primary brain cancers. Her research focus is clinical trials for nervous system tumors including tumor pharmacokinetic and pharmacodynamic investigations, imaging biomarkers and patient focused, functional endpoints. She is the Marjorie Bloomberg Tiven Professor of Neurofibromatosis and Executive Director of the Neurofibromatosis Therapeutic Acceleration Program (NTAP). NTAP accelerates effective treatments for neurofibromas via collaboration, mentorship, open and timely data sharing and streamlined research support.





Aaron Boes, MD, PhD
University of Iowa

Dr. Aaron Boes is a pediatric neurologist at the University of Iowa. He is a professor in the Departments of Pediatrics, Neurology, and Psychiatry and holds an Endowed Chair of Pediatric Neurology. He directs the pediatric neurology division as well as the UI Center for Noninvasive Brain Stimulation. A major focus of his lab is investigating the functional consequences of focal brain lesions to better understand the organization of the brain, along with research in noninvasive brain stimulation.

Amy Brooks-Kayal, MD, FANA
UC Davis School of Medicine

Dr. Amy Brooks-Kayal is professor and chair of the Department of Neurology and the Andrew John Gabor, MD, PhD, Presidential Chair in Neurology at University of California Davis School of Medicine. Clinically, she is a pediatric epileptologist. Her research focuses on the effects of epileptogenic brain injury on neuronal signaling pathways and neurotransmitter systems with particular emphasis on understanding the molecular regulation of GABA(A) receptor expression, and on development of novel therapeutics for the prevention, treatment and cure of epilepsy. Dr. Brooks-Kayal has held numerous leadership roles including as president of the American Epilepsy Society (AES), member of NINDS Advisory Council and Chair of CNNT review panel. She is currently a co-director of the Child Neurology Career Development K12 program, a member of NST-1 study section, a neurology director of the American Board of Psychiatry and Neurology and associate editor of epilepsy for the Annals of Neurology. She was the recipient of the 2019 AES Founders Award, the 2021 International League Against Epilepsy (ILAE) Ambassador for Epilepsy award and the 2023 recipient of the Child Neurology Society Bernard Sachs Award for her leading research in neuroscience relevant to the care of children with neurological disorders.



Alice Chen-Plotkin, MD, FANA
University of Pennsylvania

Dr. Alice Chen-Plotkin is the Parker Family Professor of Neurology at the University of Pennsylvania. A Phi Beta Kappa graduate and English literature major at Harvard University, she began her scientific training as a Rhodes Scholar at Oxford University. At Penn, she leads a research group studying neurodegenerative diseases and maintains a clinical practice caring for Parkinson's Disease patients. Dr. Chen-Plotkin has won top awards from the American Academy of Neurology (Jon Stolk Award, 2014), the American Neurological Association (Derek Denny-Brown Young Neurological Scholar Award, 2018), and the National Institute of Neurological Disorders and Stroke (Javits Neuroscience Investigator Award, 2024).



Catherine Chu, MD, MMSc, FANA
Johns Hopkins University

Dr. Catherine Chu is the Director of Child Neurology and Pediatric Epilepsy at Johns Hopkins University and Vice President of Neurology at Kennedy Krieger Institute. She is board-certified in Neurology (with special qualifications in Child Neurology), Epilepsy, and Neurophysiology. Dr. Chu cares for patients with refractory epilepsy and abnormal neurophysiology, specializing in developmental neurophysiology, neurosurgical planning, and responsive neuromodulation. Dr. Chu's laboratory identifies biomarkers and critical oscillations in neurodevelopmental disorders, memory and cognition, and epilepsy.



Nicole Coufal, MD, PhD, FANA
University of California, San Diego

Dr. Nicole Coufal is a physician-scientist with training in pediatric critical care and research interests in neuroimmunology. The goal of her lab is to understand the contribution of innate immunity to neurodevelopmental and pediatric neurodegenerative diseases. By understanding the interaction between the brain environment and cellular ontogeny on macrophage function, the lab strives to understand the molecular and cellular mechanisms underlying innate immune dysfunction to ultimately identify novel therapeutic targets in untreatable pediatric diseases. Her lab utilizes a combination of translational, patient-specific pluripotent stem cell and genome-wide approaches to identify cellular mechanisms in common neurodevelopmental and rare pediatric neurodegenerative disorders.



Katy Cross, MD, PhD
University of California, Los Angeles

Dr. Katy Cross is an Assistant Professor of Neurology in the Movement Disorders division at the David Geffen School of Medicine at UCLA. Her lab studies motor control in Parkinson disease and other movement disorders, with a focus on identifying neurophysiologic biomarkers that explain heterogeneity in disease features and responses to treatment. Dr. Cross sees patients with movement disorders and deep brain stimulation and her research is motivated by a desire to improve treatments and quality of life for patients.



Ethan Goldberg, MD, PhD, FANA
The Children's Hospital of Philadelphia

Ethan Goldberg, MD, PhD, FANA, is a professor in the Division of Neurology at the Children's Hospital of Philadelphia and Departments of Neurology & Neuroscience at the University of Pennsylvania. He received his MD and PhD in Neuroscience from NYU and pediatric neurology training at CHOP and Penn. He is Director of the Epilepsy Neurogenetics Initiative specializing in the diagnosis and care of children with neurodevelopmental disorders. His lab studies basic mechanisms of developmental brain disorders using human and mouse genetics, electrophysiology, human induced pluripotent stem cells, behavior, computational modeling and imaging in preclinical models.



Peyman Golshani, MD, PhD, FANA
University of California, Los Angeles

Dr. Peryman Golshani earned a Bachelor of Arts in Molecular and Cell Biology at UC Berkeley. He then obtained his MD/PhD at UC Irvine where he was mentored by the late Dr. Edward G. Jones. He completed his residency in neurology at UCLA and transitioned to an assistant professor position in 2006. He is now a professor of neurology. His laboratory focuses on understanding the neural dynamics that drive cognition and how these activity patterns go wrong in modes of autism, ID and epilepsy.



Aimee Kao, MD, PhD
University of California, San Francisco

Dr. Amy Kao is the French Foundation Endowed Professor of Neurology and Director of the UCSF MSTP. Her lab investigates basic mechanisms underlying neurodegenerative diseases, focusing on how aging, stress and gene mutations disrupt lysosomal function and protein homeostasis. She holds multiple government, private foundation and industry research grants. Her awards include the Paul Allen Distinguished Investigator Award, the Glenn Award for Aging Research and the ANA's Derek Denny Brown Young Neurological Scholar Award.



Sattar Khoshkoo, MD
Mass General Brigham/Harvard Medical School

Dr. Sattar Khoshkoo is a physician-scientist and Director of the Epilepsy Genetics Program at Mass General Brigham and Harvard Medical School. He completed medical training at UCSF, adult neurology residency at Mass General Brigham and clinical epilepsy training at Brigham and Women's Hospital and Boston Children's Hospital. After his medical training and as a research fellow at Boston Children's Hospital, he shifted his research focus to the genetic underpinnings of focal epilepsies, which he continues to work on in his lab today. Dr. Khoshkoo's lab is broadly interested in understanding the role of genetic mosaicism in normal development and in neurological diseases. He is currently in his final year of the K08 award.



Anita Koshy, MD, FANA
University of Arizona

Dr. Anita Koshy is a professor in the Department of Neurology and the Department of Immunobiology at the University of Arizona. Her clinical specialty is infections of the nervous system. The Koshy Lab studies the cellular and molecular mechanisms that enable the ubiquitous, intracellular parasite *Toxoplasma gondii* to persist in the mammalian brain.



Dimitri Krainc, MD, PhD, FANA
Northwestern University

Dr. Dimitri Krainc is the Ward Professor and Chairman of the Department of Neurology and Director of the Feinberg Neuroscience Institute at Northwestern. Previously, Dr. Krainc spent two decades at the Massachusetts General Hospital and Harvard Medical School, where he completed his research and clinical training and served on faculty. His research group has uncovered key mechanisms of neurodegeneration that have led to the development of targeted therapies. Dr. Krainc received the Javits Neuroscience Investigator Award, Outstanding Investigator Award from NIH and was elected to the Association of American Physicians, National Academy of Medicine and the National Academy of Inventors. He currently serves as President of the American Neurological Association.



Jin-Moo Lee, MD, PhD, FANA
Washington University in St. Louis

Dr. Jin-Moo Lee is the Andrew B. & Gretchen P. Jones Professor, Chair of the Department of Neurology at Washington University School of Medicine. Dr. Lee is a physician-scientist and vascular neurologist, who has dedicated his career towards understanding mechanisms underlying neurovascular injury and repair after stroke. He has published almost 300 research articles, chapters, reviews and editorials, and has been continuously funded by the NIH for more than 25 years. Dr. Lee serves on the ANA Board of Directors and the ANA's Annals of Neurology/Annals of Clinical and Translational Neurology Oversight Committee.



Thabele Leslie-Mazwi, MD, FANA
University of Washington

Dr. Thabele Leslie-Mazwi serves as chair of the Department of Neurology at the University of Washington. He is dual-trained in neurologic critical care and interventional neurology/endovascular neurosurgery. Following training, he worked at Massachusetts General Hospital as a member of the faculty of the Departments of Neurology and Neurosurgery, in the multi-specialty Neuroendovascular Group. During that time, he did extensive system-of-care work in the Northeast across six states. He left Boston to assume the Warren and Jermaine Magnuson Endowed Chair in Medicine for Neurosciences at the University of Washington. Beyond his clinical and departmental duties, he has appointments on various national committees, serves in editorial roles on several major cerebrovascular and specialty journals, and has multiple societal roles in key societies in the field. He is involved in clinical trial oversight for a variety of large, randomized, multicenter cerebrovascular trials. While his clinical research has a broad focus, it centers predominantly on acute endovascular stroke therapies. He is deeply passionate about improving care delivery across the spectrum of neurological disease.





Anli Liu, MD, MA, FANA
New York University Langone Health

Dr. Anli Liu is an Associate Professor of Neurology at NYU Grossman School of Medicine. She is a clinician-investigator with the NYU Langone Comprehensive Epilepsy Center and Center for Cognitive Neurology, Principal Investigator of the NYU Memory and Neuromodulation Laboratory, and Investigator at the NYU Neuroscience Institute. She graduated from Stanford University with a BA in Human Biology, UC Berkeley with an MA in Cognition and Education, and UCSF Medical School with a concentration in the Medical Humanities. She completed her neurology residency at NYP-Weill Cornell, and fellowships in Clinical Neurophysiology and Cognitive Neurology at Harvard-Beth Israel Deaconess.

Joanna Mattis, MD, PhD
University of Michigan

Dr. Joanna Mattis completed her education at Stanford University and an adult neurology residency and epilepsy fellowship at the University of Pennsylvania, where her research was supported by an R25 fellowship, an NINDS K08 career development award and a Taking Flight Award from CURE Epilepsy. Dr. Mattis joined the faculty at the University of Michigan in 2022 and heads an NIH-funded laboratory investigating mechanisms of the postictal state, epilepsy comorbidities and SUDEP.



Timothy Miller, MD, PhD, FANA
Washington University in St. Louis

Dr. Timothy Miller is vice chair of research for the Department of Neurology, co-director of the ALS Center and the David Clayson Professor of Neurology at Washington University School of Medicine in St. Louis. His group focuses on developing novel therapies for neurodegenerative diseases, including ALS and tauopathies. Dr. Miller has pioneered work with antisense oligonucleotides (ASOs) and has led successful ASO clinical trials in SOD1-related ALS and developed an ASO therapy for tauopathies. His work has received several distinguished awards for innovation and Dr. Miller was elected to the National Academy of Medicine in 2023.



Mercedes Paredes, MD, PhD, FANA
UC San Francisco

Dr. Mercedes Paredes is a professor in the Department of Neurology and Neuroscience, Developmental and Stem Cell Biology and Biomedical Sciences graduate programs at UCSF. Her lab focuses on identifying features of neuronal progenitor proliferation and migration that are unique to the gyrated brain, such as in humans, with an emphasis on the perinatal period.

The team combines cellular and genetic approaches to investigate the developing human brain across prenatal and neonatal stages; these ground truths are used for functional analysis in large animal models that faithfully mimic human neurodevelopment. As a physician scientist, she also works with clinical investigators to ensure patient perspectives are incorporated into research goals.





Henry L. Paulson, MD, PhD, FANA
University of Michigan

Dr. Henry L. Paulson is the Lucile Groff Professor of Neurology for Alzheimer's Disease and Related Disorders and director of the Michigan Alzheimer's Disease Center at the University of Michigan. His research and clinical interests concern the causes and treatment of various age-related neurodegenerative diseases. He is a member of the National Advisory Council for Neurological Disorders and Stroke Council at the NIH. Among his awards, Dr. Paulson is a fellow in the American Association for the Advancement of Science, member of the National Academy of Medicine, and a recipient of the Story Landis Outstanding Mentor Award from NINDS.

Annapurna Poduri, MD, MPH, FANA
Boston Children's Hospital

Dr. Ann Poduri is Director of the Epilepsy Genetics and Neurogenetics Programs, Associate Chief for Academic Development in the Department of Neurology, and the Diamond Blackfan Chair of Neuroscience Research at Boston Children's Hospital. Through her multi-disciplinary program that spans from the clinic to the laboratory, she has launched studies of that continue to reveal many genetic causes for epilepsy and other neurodevelopmental disorders, including novel discovery in the area of somatic mutation in pediatric brain disease. At BCH, she is a member of the steering committees of the Children's Rare Disease Cohorts Initiative and the Sandra L. Fenwick Pediatric Health Equity Institute. Beyond BCH, Ann serves as an elected member of the Board of the American Epilepsy Society and has served as an invited member of the Genomics Commission of the International League Against Epilepsy, Chair of the American Epilepsy Society/National Institute of Neurological Disorders and Stroke Benchmarks Stewards Committee, member of the NINDS NST1 study section, and on scientific advisory boards for companies and foundations devoted to developing precision medicine for patients with epilepsy. Her collaborative research and mentorship contributions have been recognized through numerous honors, including the American Neurological Association's Derek Denny-Brown Young Neurological Scholar Award, the American Academy of Neurology's Dreifuss-Penry Epilepsy Award, and the Harvard Club of Boston's Most Influential Women designation.



Rujuta Wilson, MD

University of California, Los Angeles

Dr. Rujuta Wilson is an Assistant Professor of Pediatric Neurology and Psychiatry at UCLA and a faculty member of the UCLA Center for Autism Research and Treatment (CART). A graduate of The George Washington University's combined BA/MD program, she completed pediatric and child neurology training at UCLA and later earned the Savant Behavioral Neurology Fellowship.



Dr. Wilson specializes in behavioral neurology, autism spectrum disorder (ASD), and related neurodevelopmental and neurogenetic conditions. Her research focuses on understanding motor abnormalities in ASD and associated genetic syndromes, using quantitative measures to identify subtle motor impairments and their developmental impact. She has also conducted research on neurofibromatosis type 1 and epileptic encephalopathies, receiving support from the Child Neurology Foundation and other research fellowships.

In addition to her clinical and research work, Dr. Wilson is deeply committed to medical education, serving as Associate Program Director of the Child Neurology Residency and Pediatric Neurology Training Director for the UC-LEND program.

William Zeiger, MD, PhD

University of California, Los Angeles

Dr. William Zeiger is an assistant professor of neurology at UCLA. He attended the University of Illinois and completed his MD and PhD at the University of Chicago. Dr. Zeiger trained in neurology at the Johns Hopkins Hospital and UCLA and completed a fellowship in movement disorders at UCLA. Dr. Zeiger specializes in the diagnosis and treatment of Parkinson's disease and atypical parkinsonian disorders. His lab researches cortical circuit dysfunction in mouse models of neurological diseases.



Common Mistakes in NIH Grant Applications

The five review criteria for most NIH grant applications are Significance, Approach, Innovation, Investigator(s), and Environment. Innovation is not necessary, but the results should have compelling significance.

Problems with Significance:

Not significant nor exciting nor new research Lack of compelling rationale Incremental and low impact research

Problems with Specific Aims:

Too ambitious, too much work proposed Unfocused aims, unclear goals Limited aims and uncertain future directions

Problems with Experimental Approach:

Inappropriate level of experimental detail Feasibility of each aim not shown Little or no expertise with approach
Lack of appropriate controls

Not directly testing hypothesis Correlative or descriptive data
Experiments not directed towards mechanisms
No discussion of alternative models or hypotheses
No discussion of potential pitfalls

No discussion of interpretation of data
Inadequate description of statistical approach/analyses

Problems with Investigator(s):

No demonstration of expertise or publications in approaches Low productivity, few recent papers
Collaborators needed but none recruited, or no letters from collaborators Inadequate funding

Problems with Environment:

Inadequate institutional support

NIH Websites

FUNDING COMPONENTS OF THE NIH

The NIH Homepage:

<https://www.nih.gov>

Homepages of the NIH Institutes, Centers & Offices:

<http://www.nih.gov/icd/>

NIH GUIDE FOR GRANTS AND CONTRACTS

Program Announcements (PAs) and

Request for Applications (RFAs):

<http://www.nih.gov/grants/guide/index.html>

NIH Grants Policy Statement:

<http://grants.nih.gov/grants/policy/>

APPLICATION PROCESS

NIH Grant Application Instructions, Guidelines and Forms:

<https://grants.nih.gov>

SF424 (R&R) Application and Electronic Submission Information (including information on new biosketch formats):

<http://grants.nih.gov/grants/funding/424/index.htm>

NIH Modular Research Grant Applications:

<https://grants.nih.gov/grants/how-to-apply-application-guide/format-and-write/develop-your-budget/modular.htm>

Standard Due Dates for Competing Applications:

<https://grants.nih.gov/grants/how-to-apply-application-guide/due-dates-and-submission-policies/due-dates.htm>

Center for Scientific Review:

<http://www.csr.nih.gov/>

NCI's Quick Guide to the Preparation of

NIH Grant Applications:

<https://deainfo.nci.nih.gov/extra/extdocs/gntapp.pdf>

NIAID Samples of grant applications & more:

<https://www.niaid.nih.gov/grants-contracts/sample-applications>

NCCIH Tips for New NIH Research Grant Applicants:

<https://www.nccih.nih.gov/grants/tips-for-new-nih-research-grant-applicants>

REVIEW PROCESS

Review Criteria for Evaluation of Research Applications:

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K - Awardee Attendee List

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2026 ANA-NINDS K-Awardee Abstracts

Autoimmune Neurology and MS

Soluble Protein Markers of Remyelination in Multiple Sclerosis

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Background: There is a critical unmet need for validated, easily accessible, soluble biomarkers that reflect remyelination in the central nervous system (CNS). Such biomarkers are essential for understanding the course of multiple sclerosis (MS), accurately assessing the efficacy of emerging remyelinating therapies, and selecting appropriate patients for remyelination trials.

Objectives: In this study, we aim to identify markers associated with effective remyelination using broad-scale proteomic discovery in samples from the ReBUILD remyelination trial. ReBUILD was a phase 2 case-crossover trial that demonstrated improvement in visual evoked potential (VEP) latency in people with MS (pwMS) treated with clemastine fumarate. We confirmed our findings in an independent transcriptomic dataset derived from MS lesions in a deeply characterized cohort from the Netherlands Brain Bank (NBB). Here, we explored the overlap between DEPs in ReBUILD and differentially expressed genes (DEGs) in remyelinating lesions in the NBB to identify proteins that reflect remyelination-related changes, rather than specific effects of clemastine.

Results: We identified numerous differentially expressed proteins (DEPs) in efficient remyelimators during and before clemastine treatment (ERclemastine) compared to suboptimal responders (SRclemastine) and corroborated their associations with capacity for remyelination in the NBB. Among those, NTRK3, a protein expressed in neurons and oligodendrocyte precursor cells, was higher in the serum of ERclemastine during treatment in ReBUILD and was upregulated in remyelinating lesions in NBB MS donors. Another CNS protein, CACNB1, was elevated in ERclemastine before treatment and upregulated in lesions with high likelihood to remyelinate in the NBB. CACNB1 also positively correlated with the active remyelination marker BCAS1. Other proteins, such as FHIT and TSC1, were lower in ER in both cohorts, and the serum relative abundance of the former showed a high predictive value for detecting future responses to clemastine (area under the curve: AUC 0.8). In ReBUILD, additional markers could also predict remyelinating capacity, such as GART, PPP3R1, and their ratio (NPXGART - NPXPPP3R1), with AUC of up to 0.91.

Conclusions: Our work identifies numerous potential markers of intrinsic and therapeutic remyelination in pwMS, and our findings can enable the discovery of novel remyelinating targets and more efficient conduct of remyelination studies.

Early Treatment Effects and Lesion Microstructural Subtypes Across the Multiple Sclerosis Disease Spectrum: A Diffusion Basis Spectrum Imaging Study

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Background: Conventional MRI cannot resolve the pathological processes coexisting within multiple sclerosis (MS) tissue. Diffusion Basis Spectrum Imaging (DBSI) decomposes the diffusion signal into anisotropic and isotropic compartments, yielding fiber, cell, and hindered ratios indexing axonal integrity, cellularity, and extracellular space. We characterized lesion microstructure across the MS spectrum and early treatment response.

Methods: Thirty-seven participants underwent multi-shell diffusion MRI: 11 healthy controls, 15 treatment-naïve relapsing MS (RMS), and 11 non-relapsing MS (NRMS; ≥ 10 -year duration, ≥ 3 years inactive). Thirteen RMS were

rescanned ~3 months after starting B-cell-depleting or sphingosine-1-phosphate receptor-modulating therapy. DBSI metrics were extracted from normal-appearing gray (NAGM) and white matter (NAWM) and lesions (subdivided into core, rim, and perilesional white matter); Gaussian mixture model clustering identified lesion subtypes.

Results: Treatment-naïve RMS showed reduced NAWM fiber ($p=0.04$) and elevated hindered ratio ($p=0.02$) versus controls. Lesions had lower fiber and cell ratios and higher hindered ratio than within-subject NAWM (all $p<0.001$). After treatment, lesional fiber ratio increased ($p=0.01$) while lesional and NAGM cell ratios declined (both $p=0.02$). Lesional cell ratio was higher in RMS than NRMS at core ($p=0.03$) and rim ($p=0.04$) but not perilesionally; treatment was spatially uniform. Clustering yielded four reproducible subtypes (edematous-hypocellular, moderately cellular, actively inflamed, structurally preserved); the inflamed subtype predominated in RMS and edematous-hypocellular in NRMS.

Conclusions: DBSI resolves microstructural lesion pathology, distinguishes relapsing from non-relapsing MS via lesion-body cellularity, and detects treatment-related changes in fiber and cell compartments. Unsupervised clustering yields reproducible lesion subtypes shifting across the disease spectrum, supporting DBSI as a candidate biomarker for in vivo lesion phenotyping.

Structure-Guided Design Of A Targeted Autoantibody Degradar For Neurologic Disease

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Abstract: Despite rapid progress in the diagnosis of autoantibody-mediated neurologic diseases, standard-of-care therapeutic options remain limited to nonspecific immunosuppression. Here, we report an alternative therapeutic strategy using targeted protein degradation to eliminate pathogenic autoantibodies while leaving the rest of the immune system intact. We previously discovered autoimmune vitamin B12 central deficiency (ABCD), a neurologic condition in which autoantibodies targeting the transcobalamin receptor (CD320) impair the transport of cobalamin (B12) from the blood into the central nervous system (CNS). Combining scanning alanine mutagenesis by phage display, cryo-electron microscopy, and computational modeling, we elucidated a highly conserved anti-CD320 epitope and defined the structural determinants of antigen-autoantibody binding. Next, we synthesized a lysosome-targeting chimera (LYTAC) comprising the lysosome targeting glycan, triGalNAc, fused to the antigenic epitope of CD320 as autoantibody bait. In vitro, this LYTAC promoted the specific lysosomal internalization and extracellular clearance of anti-CD320, restoring homeostatic cellular uptake of B12. In a passive transfer mouse model of ABCD, LYTAC treatment rapidly cleared anti-CD320 from circulation and prevented penetration of anti-CD320 into the CNS. These findings uncover the mechanism of autoantibody-antigen binding in ABCD and demonstrate targeted autoantibody degradation as a therapeutic strategy that may be generalizable to other autoimmune neurologic diseases.

Behavioral Neurology and Dementia

Interrogating Amygdala-predominant Lewy Body Disease with Spatial Proteomics

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Background: Lewy body disease (LBD) often coexists with Alzheimer's (AD), resulting in more cognitive decline than from either alone. LBD's hallmarks, asyn-positive Lewy bodies and neurites, propagate from the enteric system or olfactory bulb to the amygdala, which acts as a gatekeeper before spread to other structures. The amygdala-predominant LBD (AP-LBD) type is a special pattern of LBD that is quite prevalent in sporadic and familial early-onset AD (EOAD). Notably, AP-LBD does not conform with the most used staging systems for LBD and has received little attention. We found a link between asyn seeding-competent fibrils in cerebrospinal fluid (CSF) and the transition of LBD from the amygdala into connected regions, supporting its gatekeeper role. The factors permitting asyn propagation in the amygdala remain unknown. Our goal is to use spatial proteomics in EOAD cases with AP-LBD to identify pathways of spread and selectively vulnerable cell populations within amygdala subfields.

Methods: We identified all postmortem EOAD cases with AP-LBD from the UCSF Neurodegenerative Disease Brain Bank (n=57), 20 of which have banked CSF for asyn seeding amplification testing. We are using spatial proteomics (Nanostring GeoMx DSP with morphology, pathology, AD, and PD modules) with 50 um ROIs to select and compare neurons with phosphorylated asyn, phosphorylated tau, both, or neither. ROIs were chosen across the central nucleus, basolateral nuclei, and cortical nucleus of the amygdala.

Results: Pending analysis, we will report the proteomic differences between subfields, between different neuronal phenotypes, and their correlations to CSF seeding assay result. Of 19 CSF samples tested thus far, 53% were positive for asyn seeding; an additional sample is pending.

Conclusions: Proteomic variation among amygdala subfields and distinct neuronal phenotypes may uncover molecular determinants of selective neuronal vulnerability, deepening our understanding of AP-LBD pathobiology and facilitating the development of targeted diagnostic and therapeutic interventions.

Neural Replay Events Emerge During Periods of Cortical Beta Suppression

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Abstract: Neural replay, temporally compressed reactivations of task-related activity during rest¹, has emerged as a candidate mechanism for stabilizing newly acquired skills^{2–4}. However, the oscillatory conditions that allow replay to emerge in humans is unknown. In particular, whether neural replay events are linked to specific brain oscillatory states remains unclear. Here we test the hypothesis that replay emerges preferentially during transient beta-band oscillatory suppression, a neural state that is associated with increased plasticity and learning^{5–9}.

We analyzed magnetoencephalography (MEG) data from a repository of 31 participants performing a well-characterized motor sequence learning task in which 10-second bouts of sequence typing alternated with 10-second rest periods^{4,10}. We implemented a previously described neural replay detection pipeline^{4,11}. To characterize oscillatory dynamics around replay, source-level MEG data were bandpass filtered in the beta range, Hilbert transformed, and epoched trial-by-trial from 1 s before to 1 s after replay onset. Beta power surrounding replay was examined relative to rest baseline and compared with matched randomly selected rest time points.

Replay events were associated with a transient reduction in beta power surrounding event onset. At the group level, replay-locked averaging revealed a clear beta power decrease preceding replay onset, with a second drop centered near the replay event. This replay-related beta profile was not observed for randomly selected control periods, suggesting that the effect was not explained by fluctuations in rest-related beta activity.

These findings provide evidence that human motor replay is embedded within a low-beta state and suggest that transient beta suppression may mark a cortical state that facilitates replay. This aligns with recent animal literature showing that elevated beta-band bursting competes with the neural replay events that are causal drivers of performance gains⁹.

These results identify beta dynamics as a candidate target for testing how oscillatory brain state shapes offline motor learning.

β -Amyloid Induces Microglial Expression GPC4 And APOE Leading to Increased Neuronal Tau Pathology and Toxicity

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Background: Heparan sulfate proteoglycans (HSPGs) are emerging as critical regulators of protein aggregation and intercellular propagation in neurodegeneration, yet their cell-type-specific regulation and function in Alzheimer's disease (AD) remain incompletely defined. Here, we identify a proteoglycan remodeling program in microglia triggered by β -amyloid (A β) pathology.

Methods: Human iPSC-derived microglial models were used to examine proteoglycan remodeling following exposure to fibrillar A β . Surfaceome profiling using HRP-WGA-based proximity labeling identified cell-surface proteins selectively altered by amyloid pathology. Functional studies in iPSC models assessed the role of Glypican 4 (GPC4) in tau aggregate binding, uptake, trafficking, and seeding. Pathological relevance was evaluated in human AD brain tissue, and in vivo functional validation was performed using *Drosophila* models of amyloid- and tau-associated neurotoxicity.

Results: Surfaceome profiling of human iPSC-derived microglia reveals that fibrillar A β selectively induces the upregulation of multiple HSPGs, with prominent enrichment of the glypican family member Glypican 4 (GPC4), a GPI-anchored HSPG not previously linked to microglial biology. A β fibrils drive a robust increase in microglial cell-surface heparan sulfate and GPC4 expression, a response not recapitulated by monomeric peptides or canonical inflammatory stimuli, indicating an amyloid-specific glycan remodeling program. Functionally, GPC4 acts as a key determinant of proteopathic cargo handling via their heparan sulfate chains, facilitating tau aggregate binding, uptake, and intracellular trafficking. Proteolytic shedding of GPC4 further generates a soluble HSPG pool capable of acting in

trans to enhance tau internalization into neighboring cells. Mechanistically, GPC4 cooperates with the heparan sulfate-binding protein APOE to amplify tau uptake and seeding, suggesting the formation of a proteoglycan-centered extracellular complex that promotes proteopathic spread. In vivo, glial expression of the GPC4 ortholog exacerbates amyloid- and tau-associated toxicity, while in human AD brain, microglial GPC4 is enriched in peri-plaque regions and strongly associates with local neuritic tau pathology.

Conclusions: Together, these findings position microglial HSPG remodeling as a mechanistic link between amyloid deposition and tau propagation. This work highlights proteoglycans, particularly GPC4, not merely as passive scaffolds, but as active regulators of proteopathic signaling, uptake, and intercellular spread in neurodegenerative disease.

Tau Aggregation Triggers Mislocalization of The RNA Binding Proteins PNN And RNPS1 Leading to Aberrant Splicing and Neurotoxicity

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Background: Tauopathies are progressive neurodegenerative conditions characterized by the accumulation of aggregated tau protein in selectively vulnerable neurons that are lost early in the course of these diseases. Recent work has shown that RNA and RNA binding proteins may facilitate tau aggregation, and aberrant RNA splicing has been detected in human tauopathy subjects. However, the underlying cause of these splicing-related changes, and how they are linked to tau aggregation and neurodegeneration in tauopathies is not well understood.

Methods: To study mechanisms of tau aggregation and neurotoxicity, we produced a novel induced pluripotent stem cell (iPSC) model that stably expresses a mutant form of full-length tau fused to FRET-compatible fluorophores. We induced tau aggregation in this control iPSC line by transducing tau aggregates derived from different human tauopathies, followed by FACS-sorting FRET-positive iPSCs. Upon differentiation into excitatory glutamatergic neurons, control cells contain only soluble tau, whereas tau aggregate iNeurons contain hyperphosphorylated, insoluble tau fibrils.

Results: Using this iNeuron model, we found that tau aggregation induces the mislocalization of several RNA binding proteins (RBPs) to cytoplasmic tau aggregates, including the splicing-related protein pinin (PNN) and its binding partner RNPS1. To test the role of RBPs in tau aggregate neurotoxicity, we performed an unbiased, high throughput iNeuron CRISPRi survival screen targeting over 1900 RBPs. This screen identified PNN knockdown to be one of the most selectively toxic hits in tau aggregate iNeurons, but was only mildly toxic to control iNeurons. This toxicity was linked to reduced nuclear PNN and increased aberrant splicing and transcriptomic changes in tau aggregate iNeurons compared to control iNeurons. To test whether tau aggregation induces mislocalization and nuclear depletion of PNN in human tauopathies, we next performed in-depth immunohistochemistry (IHC) analysis of PNN and RNPS1 in the middle frontal gyrus of human post-mortem tissue across several control (4), AD (13), and PSP (7) cases. In human control subjects, PNN was present in the majority of neurons across most cortical layers, with the highest nuclear levels present in large layer III, V, and VI neurons. Neuronal nuclear PNN levels were significantly reduced in AD, and both PNN and RNPS1 frequently mislocalized to tau aggregate containing neurons in AD and PSP.

Conclusions: Together, this work suggests PNN mislocalization to cytoplasmic tau aggregates may underlie the aberrant splicing and neurotoxicity observed in human tauopathies. Future work will elucidate whether aberrant transcripts induced by reduced nuclear PNN can serve as biomarkers of PNN dysfunction in human tauopathy subjects.

A β Aggregates Bind the U1 Spliceosomal Ribonucleoprotein in Alzheimer Disease Brain

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Abstract: Missplicing due to U1 small nuclear ribonucleoprotein (U1 snRNP) insolubilization and dysfunction has been identified in Alzheimer disease (AD) brain. Cytoplasmic aggregation and mislocalization of the U1 snRNP partly co-localizes with tau neurofibrillary tangles, and some evidence for tau-U1 binding has been identified. However, tau-U1 co-localization by immunohistochemistry is only partial, and insoluble U1 small nuclear RNA (snRNA) binding proteins correlate better with A β than with tau in unbiased proteomics. While investigating the sedimentation characteristics of A β aggregates capable of diffusing out of AD brain tissue, we unexpectedly found that some were bound to small RNA. A β immunoprecipitation and deep sequencing revealed the U1 snRNA, with specific binding confirmed through reverse immunoprecipitation and oligonucleotide hybridization. Double immunoelectron microscopy revealed decoration of A β fibrils, more than tau fibrils, with the U1-70k protein, a component of the U1 snRNP. Immunofluorescence of unfixed cryostat sections but not formalin fixed paraffin embedded sections of AD brain revealed labeling of a subset of amyloid plaques with anti-U1-70k antibodies, confirmed by RNA in situ hybridization of the U1 snRNA. We conclude that a subset of AD brain A β aggregates are bound to the U1 snRNP at the edges of some amyloid plaques, explaining prior proteomics findings. These data provide a potential link between A β aggregation and spliceosome dysfunction and unite A β with other fibril-forming proteins across the neurodegenerative

diseases whose aggregation is affected by RNA binding.

Cerebrovascular Disease

Deletion of Astrocyte-Specific Toll-Like Receptor 4 Signaling is Cerebroprotective following Focal Cerebral Ischemia

Presenting Author: *Bolanle Famakin, MD, University of Wisconsin, Madison, WI*

Co-Authors: *Joseph E. Giron, BS, University of Wisconsin, Madison, WI, Cetin Genc, MD, PhD, Brigham and Women's Hospital, Boston, MA, Bilgehan Solmaz, MD, PhD, Istanbul Training and Research Hospital, Istanbul, Turkey*

Background: The contribution of astrocytic Toll-like receptor 4 (TLR4) signaling to blood-brain barrier (BBB) dysfunction and ischemic brain injury remains incompletely understood despite increasing evidence linking innate immune activation to stroke progression. While previous studies have shown that global TLR4 deficiency is neuroprotective following experimental stroke, the cell-specific mechanisms underlying this effect remain largely unknown. We hypothesized that the cerebroprotective effect of global TLR4 deficiency in focal cerebral ischemia may be cell-specific. We evaluated the effects of astrocyte-specific and endothelial-specific TLR4 deletion on neurovascular injury following transient middle cerebral artery occlusion (MCAO).

Methods: Tamoxifen-inducible conditional TLR4 deletion was generated using Aldh1/1-creERT2/TLR4flox/Ai14 mice to target astrocytes and Smoc-creERT2/TLR4flox/Ai14 mice to target endothelial cells. Cre-negative littermate controls were studied in parallel. BBB dysfunction was assessed using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI). Area under the curve (AUC) and time-to-peak (TTP) measurements were used to evaluate BBB permeability and perfusion abnormalities. Infarct volume was quantified during the acute and subacute phases following cerebral ischemia.

Results: Astrocyte-specific TLR4 deletion altered the response to cerebral ischemia. During the acute phase, astrocyte-specific TLR4 knockout mice exhibited significantly reduced infarct volumes compared with wild-type controls ($19.83 \pm 2.80\%$ vs $37.47 \pm 4.91\%$; $p=0.0118$). This protection persisted into the subacute phase, with infarct volumes remaining significantly reduced in astrocyte-specific TLR4 knockout mice compared with controls ($9.8 \pm 5.3\%$ vs $30.3 \pm 2.5\%$; $p=0.043$). In contrast, endothelial-specific TLR4 deletion did not confer comparable protection and demonstrated a trend toward larger infarct volumes compared with controls ($39.22 \pm 9.89\%$ vs $16.56 \pm 5.68\%$; $p=0.1063$). MRI-derived BBB permeability metrics were not significantly altered by genotype in either model (all $p>0.05$).

Conclusions: These studies identify astrocytic TLR4 signaling as an important mediator of ischemic brain injury following cerebral ischemia and provide a potential cellular mechanism for the neuroprotection previously observed in global TLR4 knockout mice. Importantly, endothelial-specific TLR4 deletion did not confer comparable protection, suggesting that astrocytes may represent a critical cellular source of TLR4-mediated injury after stroke. The sustained reduction in infarct volume following astrocyte-specific TLR4 deletion supports astrocytic TLR4 signaling as a promising therapeutic target for limiting infarct progression and improving recovery after ischemic stroke.

Transcriptomic Profiling of Stroke Thrombi to Resolve Ischemic Stroke Etiology

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Background: Identifying the etiology of large vessel occlusion stroke is essential for secondary prevention, yet 20 to 30% of strokes remain cryptogenic, and histologic clot composition overlaps substantially across causes. Transcriptomic approaches may resolve cellular and molecular differences that histology cannot.

Methods: We performed single-cell RNA-sequencing on thrombi retrieved at mechanical thrombectomy, profiling 100,436 cells across 17 samples and retaining 12 high-quality samples after quality control, grouped by clinical cause (cardioembolism due to atrial fibrillation, and carotid atheroembolism). To extend these findings at scale, we performed bulk RNA-sequencing on an expanded cohort of thrombi spanning atrial fibrillation, carotid atherosclerosis, and additional determined and cryptogenic etiologies, with paired circulating leukocytes analyzed alongside clinical covariates.

Results: Profiling revealed etiology-associated transcriptional programs, including distinct macrophage gene expression between carotid atherosclerosis and atrial fibrillation clots and upregulated neutrophil extracellular trap signaling across cell types in atrial fibrillation clots. Bulk RNA-sequencing recapitulated these etiology-associated signatures and extended them across additional stroke causes.

Conclusions: Single-cell and bulk transcriptomic profiling of stroke thrombi identify reproducible, etiology-associated molecular signatures, supporting their development as biomarkers of stroke etiology with potential to address cryptogenic stroke.

Single-Cell RNA Sequencing Identifies an Ischemia-Specific Immune Transcriptome in

Human Acute Ischemic Stroke due to Large Vessel Occlusion

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Background: Acute ischemic stroke due to large vessel occlusion (AIS-LVO) remains one of the most devastating stroke subtypes, with poor outcomes persisting despite advances in reperfusion therapies. This variability in recovery highlights a gap in current AIS management, which primarily focuses on restoring blood flow but does not directly address the downstream molecular events of the ischemic cascade—including excitotoxicity, oxidative stress, inflammation, blood-brain barrier disruption, and apoptosis—that contribute to ongoing neuronal injury. Early transcriptional responses following ischemic stroke provide a critical window into the molecular programs that determine whether injured brain tissue recovers or progresses to irreversible infarction. The Transcriptomic Inflammatory Biomarkers of Large Artery Stroke (TRAILBLAZE) study leverages peri-infarct endovascular sampling and single-cell RNA sequencing (scRNA-seq) to characterize early human cellular and molecular responses during AIS-LVO. By directly sampling hypoxic arterial blood distal to the occlusion during active ischemia, TRAILBLAZE aims to validate candidate pathways identified in preclinical models while uncovering novel human-specific mechanisms that may inform future cerebroprotective therapies.

Methods: TRAILBLAZE is a prospective observational study of patients ≥ 18 years old presenting to Washington University School of Medicine with anterior circulation AIS-LVO treated with endovascular thrombectomy (EVT) within 24 hours of last known well (LKW). During EVT, paired arterial blood samples were collected: (1) at groin puncture and (2) distal to the occlusion following microcatheter traversal through the thrombus. Buffy coats were isolated using a specialized cooling and storage protocol designed to preserve cell integrity for subsequent batch processing. Whole cell single-cell transcriptional profiling was performed using the 10x Genomics Chromium Next GEM-X Single Cell 5' platform, followed by sequencing on the Illumina NovaSeq X Plus (~50,000 reads per cell; ~15,000 cells per sample). Sequence alignment and gene quantification were performed using Cell Ranger. Downstream analysis was conducted in Seurat and included stringent quality control filtering based on gene and UMI counts, mitochondrial RNA proportion, and putative doublets. Cell types were annotated using canonical marker genes and reference-based methods. Differential gene expression analysis was performed using the MAST hurdle model, adjusting for patient ID, batch effects, and cellular detection rate. P-values were corrected using the Benjamini-Hochberg method ($FDR < 0.05$), with additional filtering for absolute $\log_2$ fold-change ≥ 0.25 . Gene ontology (GO) enrichment analysis identified overrepresented biological pathways among differentially expressed genes.

Results: A proof-of-concept analysis was performed on the first seven patients enrolled in TRAILBLAZE (mean age 67 years [SD 16.4], 57% male, mean LKW-to-groin puncture time 488 minutes). scRNA-seq yielded 97,137 cells across six major cell populations: B cells, T cells, monocytes, natural killer cells, erythrocytes, and platelets. Although cellular composition was similar between paired samples, post-thrombus blood demonstrated distinct transcriptional signatures, with 45,779 upregulated and 8,437 downregulated genes identified. CD14⁺ monocytes represented the most transcriptionally active cell population and demonstrated increased expression of genes associated with innate immune regulation, GTPase-mediated signal transduction, myeloid differentiation, cytoskeletal remodeling, and regulation of T-cell activation. Across multiple cell types, there was broad downregulation of pathways involved in ribosome biogenesis, rRNA processing, ATP biosynthesis, and global protein synthesis. In parallel, pathways related to histone modification, RNA catabolism, and immune cell differentiation were upregulated.

Conclusions: These findings suggest that hyperacute ischemic stroke induces coordinated metabolic and immune reprogramming within post-thrombus arterial blood cells, characterized by suppression of energy-intensive biosynthetic processes alongside activation of adaptive cellular stress responses. Furthermore, CD14⁺ monocytes appear to adopt a distinct vascular-interface immunoregulatory phenotype marked by controlled innate immune sensing, active trafficking, and early adaptive immune signaling. Although preliminary, these results establish a novel human model for interrogating ischemia-induced molecular responses during AIS-LVO. This approach may help overcome longstanding translational barriers in cerebroprotective drug development by identifying clinically relevant human-specific pathways and therapeutic targets during the earliest stages of ischemic injury.

Using Machine Learning to Combine Routinely Collected Prehospital Clinical Data with The Face-Arm-Speech Neurological Examination for Large Vessel Occlusion Stroke Triage Testing

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Background: Prehospital triage of suspected large vessel occlusion (LVO) stroke patients to thrombectomy centers is recommended, but existing tests result in many false positives and negatives. Most existing prehospital triage tests focus on the neurological examination and do not incorporate routinely collected prehospital data. Using machine learning to incorporate these variables has improved diagnostic performance for LVO stroke in Japan. We sought to explore whether a

similar multi-variable machine learning approach would improve LVO stroke diagnostic performance among prehospital suspected stroke patients in the United States.

Methods: The 2018 ESO Research Dataset includes patient care records from adult prehospital suspected stroke patients and hospital-reported ICD-10 diagnostic codes. The Face-Arm-Speech stroke neurological examination, which has been implemented as an ordinal LVO test (0-3), was the baseline test to improve upon. Samples without complete Face-Arm-Speech data were excluded. Additional predictor variables included age, sex, heart rate, systolic and diastolic blood pressures, respiratory rate, oxygen saturation, glucose, and GCS total score. The reference standard of LVO stroke was identified using ICD-10 codes indicating a stroke due to an abnormality of a carotid, middle cerebral, or basilar artery (e.g. I63.51). Analyses were performed using scikit-learn (version 1.7.2). A 70/30 stratified train-test split was used to preserve class balance. Stratified 5-fold cross-validation among training data evaluated multiple models. RandomizedSearchCV was used to explore the variable and hyperparameter spaces. The average precision score guided model optimization and selection. Binary thresholds were then tuned to target the sensitivity closest to 80% that provides the highest Youden's Index (sensitivity + specificity - 1).

Results: 3074 patient care records were eligible. The train dataset included 2141 participants, of whom 249 (12%) had LVO stroke. Cross-validation on the training data set resulted in Youden's Index values of: XGBoost 0.24, Random Forest 0.20, Logistic Regression 0.19, Face-Arm-Speech (baseline) 0.19, and multilayer perception 0.17. Sensitivities were 80% by design, except for Face-Arm-Speech ≥ 2 (81% sensitive). Specificities and positive predictive values were: XGBoost 0.43/0.16, Random Forest 0.40/0.15, Logistic Regression 0.38/0.15, Face-Arm-Speech (baseline) 0.38/0.15, and multilayer perception 0.37/0.14.

Conclusions: Adding demographics, vitals, glucose, and GCS to Face-Arm-Speech using multiple machine learning models resulted in at most a 5% increase in Youden's Index (XGBoost). This is not likely a large enough increase to justify the added complexity of machine learning, especially given the simplicity of Face-Arm-Speech alone and persistently low positive predictive values (<20%). Next steps include adding approximately 80,000 patient care records (dataset years 2019-2024), further refinements via mentored feedback on preprocessing, variable selection, hyperparameter tuning, and multi-class classification. Incorporation of second-generation LVO stroke scales (LAMS, VAN, RACE, and others) is planned; however, sample sizes will be much smaller due to limited adoption in the United States. The final test dataset evaluation will occur after the training dataset pipeline has been fully optimized.

Impact Of Cerebral Acid-Base Status on Spreading Depolarization

Presenting Author: *Britta Lindquist, MD, PhD | University of California, San Francisco*

Background: Fifteen to 20% of acute ischemic stroke patients are admitted to the intensive care unit (ICU), often requiring endotracheal intubation and mechanical ventilation. Current clinical guidelines make no recommendations regarding pH, PCO₂, or HCO₃⁻ goals in patients with acute ischemic stroke. Acid-base management strategies may exacerbate or mitigate cerebral injury following stroke: metabolic or respiratory acidosis inhibit the initiation and propagation of spreading depolarizations (SDs), which are excitotoxic waves of cellular depolarization that promote the expansion of ischemic strokes in vivo. The involvement of intracellular vs extracellular targets has not yet been clarified.

Methods: I used acute brain slices to examine the effects of tissue acidification on SD without confounding effects from variable cerebral blood flow. I freshly prepared acute brain slices (coronal, 350um thick) from C57Bl/6J mice of both sexes. To manipulate metabolic acid-base status of both intra- and extra-cellular compartments, I manipulated the HCO₃⁻-buffer content of artificial cerebrospinal fluid (pH 6.6 – 8.0), bubbled with 5% CO₂-95% O₂, replacing sodium bicarbonate with equimolar sodium methylsulfate to maintain iso-osmolarity, iso-natremia and iso-chloremia. To selectively manipulate extra-cellular pH, I applied HCO₃⁻/CO₂-free HEPES-buffered artificial cerebrospinal fluid (pH 6.6 – 7.6), bubbled with 100% O₂. I induced SDs by micro-injection of KCl (1M) and obtained recordings of intrinsic optical signals and extracellular field potentials from stratum radiatum of the hippocampal CA1 region.

Results: In HCO₃⁻-buffered aCSF, KCl reliably elicited SD over the bath pH range of 6.8 – 8.0. SD could not be elicited at pH 6.6. Propagation rate followed a sigmoidal dose-response relationship (bath pH₅₀ ~7.24), with minimum propagation rate 2.2 mm/min in acidemic bath solution (pH 6.8) and maximum propagation 7.4 mm/min in alkalotic bath solution (pH 7.8). In HEPES-buffered aCSF, SD was elicited at all pH levels tested (6.6 – 7.6). Minimum propagation rate 1.6 mm/min was observed in acidemic bath solution (pH 6.6) and maximum propagation rate 2.8 mm/min was observed in alkalotic bath solution (pH 7.6). The dose-response relationship was incompletely characterized by the effective pH range of HEPES, with pH₅₀ estimated as ~6.3.

Conclusions: SD propagation is modulated by parenchymal and extracellular acid-base status. Application of HCO₃⁻-CO₂-buffered artificial cerebrospinal fluid is more effective than HEPES-buffered solution to modify SD propagation rate, suggesting that intracellular as well as extracellular targets are involved. In future studies, I will selectively acidify and alkalinize intracellular compartments with sodium propionate and ammonium chloride, and I will evaluate additional downstream mediators of cerebroprotection by acid-base status, including cerebral blood flow, ion channel modulation, and cellular energy phenotypes.

Selective Hippocampal Neuronal Loss Following Neonatal Hypoxia-Ischemia

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Background: Hypoxic-ischemic encephalopathy remains a major cause of neonatal mortality and long-term neurological disability, with limited treatment options beyond therapeutic hypothermia. Advancing effective treatments requires a deeper understanding of the complex and dynamic biological responses to hypoxic-ischemic brain injury (HI). We used Sequential Fluorescence In-Situ Hybridization (SeqFISH) to map spatial gene changes after neonatal HI in our murine model.

Methods: Neonatal HI was induced at postnatal day 10 by permanent coagulation of the left common carotid artery (ischemia), 1-hour recovery, and 10% O₂/90% N₂ for 50 minutes at 37 °C (hypoxia). Brains were collected at multiple post-injury time points, sections from OCT-embedded frozen brains were processed on the Spatial Genomics Gene Positioning System (GenePS) using the Mouse Brain Mapper Panel supplemented with ARG1, ARG2, fibrosis-related (TGFB1, COL1A2) and efferocytosis-related genes (Rac1, STAT6). Images were analyzed using Spatial Genomics Navigator, followed by clustering and differential expression analysis in MATLAB.

Results: The hippocampal neurons were among the most significantly injured cells by HI insult. These neurons formed Cluster 10 and spatially located to CA1-3 of the hippocampus. At Day 1 HI, these cells display prominent Rac1/STAT6 induction, as well as COL1A2 compared to sham mice, indicating early activation of efferocytotic pathways and extracellular matrix remodeling. By Day 5 HI, there is increased expression of ARG1, IFNGR1, and TGFB1, suggestive of activation of pro-repair pathways, while sham mice expressed ARG2, suggesting different functions of arginase isoforms.

Conclusions: Our preliminary data suggest early activation of efferocytosis and extracellular matrix remodeling pathways in neonatal hippocampus after HI. Further studies are necessary to validate the mechanistic pathways identified in our study.

Epilepsy

Altered Sleep Microarchitecture in Epilepsy: Spindles, Slow Oscillations and Phase Precession of Spindle-SO Coupling

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Background: Spindles (11-16 Hz) and slow oscillations (0.5-1.5 Hz; SO) are fundamental elements of the NREM sleep microarchitecture, often co-occurring in a phase-dependent manner. This cross-frequency coupling is critical for the temporal coordination of neural activity in sleep and for memory consolidation. We previously showed that reduced spindle activity and spindle-SO coupling is associated with lower cognitive performance in temporal lobe epilepsy (TLE). In this study, we present evidence for a novel spatiotemporal relationship between spindles and SO, termed phase precession, and then compare the properties of this relationship across the lifespan and between control participants and those with TLE.

Methods: We studied 101 participants free of major neurological disease, and 111 participants with TLE, ranging in age from 20 to 85 years. Continuous overnight scalp EEG was sleep-staged, and spindles and SO were detected, using previously-published algorithms. The SO phases corresponding to spindle detections were plotted against the anterior-posterior scalp position. Circular-linear statistics were then used to determine the best fit line, and quantify the slope, strength (R value), and significance. Linear and logistic regression models were used to test the association of these phase precession parameters with demographics and group.

Results: Visual inspection of circular phase plots across the brain topography revealed a striking pattern. There was a systematic shift in phase, with spindles occurring at sequentially earlier SO phases relative to their anterior-posterior position. Phase-position plots confirmed this relationship at both an individual and group level. Examining phase precession properties across demographics, we found that older age is associated with reduced phase precession strength ($F=5.15$, $b=-0.001$, $R=0.37$, $p=0.008$), and a flattening of the slope ($F=2.64$, $b=-0.005$, $R=0.23$, $p=0.016$). In contrast, we did not find any significant differences between male and female sex ($p>0.05$). Comparing TLE to control participants, we found a significant reduction in phase precession strength, on average by 25.7% ($F_{\text{model}}=6.36$, $t=3.96$, $p=0.0001$, adjusting for age, sex, and total spindle count). Concordant with this finding, a smaller proportion of TLE patients had significant phase precession (Cntrl 91% vs TLE 74%; $\text{Chi}^2_{\text{model}}=13.7$, $p=0.019$).

Conclusions: We report a novel spatiotemporal organization of spindle-SO coupling, characterized by a systematic precession of the SO phase of spindles along the anterior-posterior axis of the brain. The integrity of phase precession degrades with advancing age and is reduced in patients with temporal lobe epilepsy. We propose that phase precession of spindle-SO coupling may serve an important mechanism for how information processing is coordinated across the brain in sleep, and how it may be impaired in disorders such as epilepsy.

Hippocampal-cortical Mechanisms of Altered Memory Consolidation in Epilepsy

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Abstract: Temporal lobe epilepsy (TLE) is the most common epilepsy in adults, affecting more than 50 million worldwide. Memory impairment is a major associated morbidity of TLE and affects 70% of people with TLE. While the cellular mechanisms necessary for memory formation in health are beginning to be understood, how these circuits are disrupted in TLE is relatively less known. In this project, I record single unit firing from the hippocampus and prefrontal cortex during early learning of a spatial navigation task, using a rodent model of temporal lobe epilepsy. My preliminary findings suggest disruption of hippocampal-level circuitry at the level of sharp-wave ripples and downstream formation of cortical memory ensembles.

The Effects of Single Pulse Electrical Stimulation on Intracranial EEG Monitoring

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Background: Cortico-cortical evoked potentials (CCEPs) are cerebral waveforms elicited by single-pulse electrical stimulation (SPES). These waveforms can be used to evaluate brain networks during intracranial EEG monitoring (iEEG). While generally considered to be a safe procedure, it is not known what effects SPES may have on subsequent EEG recording in the epilepsy monitoring unit (EMU).

Methods: A cohort of 28 patients underwent SPES at the beginning of iEEG monitoring. A control group of 28 patients was matched for age and baseline seizure frequency. We compared the duration of EMU stay, time until first seizure, and the number of spontaneous seizures. We compared the spike rate one hour pre- and post-SPES. Lastly, in a subgroup of 14 patients we compared functional connectivity in 10-minute segments pre-SPES, immediately post-SPES, and 50 minutes post-SPES.

Results: There was no significant difference between SPES and controls in median time until first seizure (25.7 hrs. vs. 20.5 hrs.). There was a nonsignificant trend towards longer implantation in the SPES group compared with the control group (median hrs. 204.5 vs. 166.5, respectively, $p = 0.056$). There were significantly more subclinical seizures in the SPES group (median 7 vs 0, respectively, $p = 0.011$), but no difference in total seizures. A general linear model comparing SPES, baseline seizure frequency, anti-seizure medication (ASM) wean rate, mean ASM dose, epilepsy duration, and seizures induced with SPES demonstrated that only the ASM wean rate and mean dose of ASM significantly correlated with the duration of EMU monitoring ($P < 0.05$ for both, partial η^2 0.198 and 0.21, respectively, adjusted $R^2 = 0.514$); however, both were inversely correlated ($\beta = -0.029$ and -0.02 , respectively). A similar model showed that SPES and the mean ASM dose were significantly correlated with total seizures ($P < 0.05$, partial η^2 0.102 and 0.085, respectively, adjusted $R^2 = 0.207$). The model for time until first seizures was only significant for mean ASM dose ($P < 0.001$, partial η^2 0.324, adjusted $R^2 = 0.345$). There was no difference in spike rate pre- vs post-SPES (mean spike rate 31.66 spikes/min vs 29.46 spikes/min, respectively, $P = 0.55$). Of the 14 patients with connectivity analyses, 8 showed temporary increase, 5 showed temporary decrease, and 1 had no significant change in connectivity post-SPES (KS test, $p < 0.05$). However, no significant median connectivity differences were found after SPES (Fig. 1A, KW test, $p = 0.9871$). The median from the mean connectivity difference matrices (pre-SPES – post-SPES first 10 minutes & pre-SPES – post-SPES last 10 minutes) were computed across patients (Fig. 1B). Distributions were centered around 0, indicating no net change following SPES. There was a small but significant increase in seizure onset zone (SOZ) nodal efficiency, node strength, and eigenvector centrality with a decrease in betweenness in the last 10 minutes in the post-SPES hour compared to first 10 minutes (Fig. 2, t test, $P < 0.05$); however, the initial 10 minutes were similar to baseline, suggesting that the later change could be related to other factors.

Conclusions: Overall, our study demonstrates that SPES at the beginning of iEEG monitoring was correlated with increased seizures, but otherwise does not significantly alter the clinical monitoring nor does it produce significant changes in epileptogenicity or functional network connectivity, supporting its use in clinical or research practice.

Circadian Clock Neurons Time Seizures in Drosophila

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Background: People with epilepsy experience daily oscillations in seizure risk.

Methods: Using a video-tracking model to automate detection, we explored mechanisms underlying spontaneous seizures in *Drosophila*.

Results: We find that flies, like people, exhibit daily oscillations in seizure risk. These rhythms are absent in flies lacking a molecular circadian clock and are independent of sleep. Thermogenetic screening and cell-specific clock perturbations reveal that the ventrolateral neurons, including “morning cells”, and dorsolateral neurons, comprising the “evening cells”, drive diurnal oscillations in seizure risk. Pigment-dispersing factor and short neuropeptide F from morning cells increase seizure risk during the day. During this time, morning cell activity increases whole-brain activity. At night, morning cells switch to glycine signaling likely acting on evening cells to suppress seizures. Important from a therapeutic perspective, optogenetic inhibition of clock neurons suppresses seizure risk.

Conclusions: Our findings reveal an unexpected role for brain clock neurons in controlling daily seizure risk.

Multisite Resting-State fMRI Reveals Network Segregation Loss and Lateralized Connectivity Changes in Epilepsy

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Background: Interictal functional aberrations in focal and generalized epilepsies can be measured using resting-state fMRI, and changes in canonical cortical networks and subcortical structures have been reported. However, the spatial extent and network specificity of these changes remain poorly characterized, partly due to limited sample sizes in single-site studies. We aimed to address this gap by creating a centralized, multisite dataset within the ENIGMA-Epilepsy Consortium and applying a network-informed, region-of-interest (ROI)-based functional connectivity (FC) analysis. We quantified relative network segregation and integration by characterizing the number of strong ROI-to-ROI connections within and between canonical networks.

Methods: We assembled data from 934 participants across 10 sites: healthy controls (HC, n=360), focal epilepsy (FE, n=479), and generalized epilepsy (GE, n=95). Images were preprocessed with fMRIPrep. Regional time series were extracted from 432 atlas-defined ROIs, combining the Schaefer 400x7 cortical atlas with 32 Melbourne subcortical ROIs. Participant-level FC matrices were calculated at a 10% proportional threshold. For each ROI, FC degree was defined as the number of suprathreshold edges. To test network-level hypotheses, degree was calculated as global, within-network, and between-network degree. Within-network degree was defined as edges between nodes in the same Yeo-7 network; between-network degree excluded within-network connections. We tested three prespecified contrasts: 1) FE vs GE vs HC; 2) left-onset FE (LFE, n=200) vs right-onset FE (RFE, n=153) vs HC; and 3) left TLE (LTLE, n=183) vs right TLE (RTLE, n=140) vs HC. ROI-wise ANCOVA models included age, sex, site, mean framewise displacement, and scan duration as covariates. Pairwise comparisons used Welch's t-tests on covariate-adjusted residuals. Results were FDR-corrected at $q < 0.05$, and Cohen's d was reported as the effect size.

Results: Consistent with our primary hypothesis, epilepsy was associated with widespread loss of network segregation. In the FE vs GE vs HC analysis, significant group effects were observed for within-network degree in 120/432 ROIs and for between-network degree in 50/432 ROIs. Compared with HC, both FE and GE showed reduced within-network degree concentrated in associative systems, particularly default mode and salience networks, together with increased between-network degree involving sensorimotor and subcortical regions. In GE, within-network reductions were prominent in temporal default mode and salience networks, involving 92 ROIs with effect sizes up to $|d| = 0.61$. GE also showed increased between-network degree in somatosensory regions (6 ROIs, $|d|$ up to 0.47) and in subcortical anterior caudate and dorsal-anterior thalamic ROIs. In FE, within-network reductions versus HC were widespread, with 77/83 significant ROIs showing decreased within-network degree and effect sizes up to $|d| = 0.47$. These abnormalities were concentrated in salience, default mode, and visual networks. FE also showed increased between-network degree in sensorimotor regions (14 ROIs, d up to 0.46) and dorsal-anterior thalamus ($d = 0.22$). In FE laterality analysis, RFE showed broader within-network reductions than LFE (33 ROIs, with peak $|d| = 0.64$, and 16 ROIs, with peak $|d| = 0.59$, respectively). These abnormalities were centered on ipsilateral temporal default mode and frontal salience cortex, supporting a lateralized focal network signature. The TLE-only analysis showed larger peak effect sizes with the same pattern, despite smaller subgroup sizes, with RTLE reaching $|d| = 0.69$ and LTLE reaching $|d| = 0.62$. Eight ROIs directly distinguished TLE laterality groups.

Conclusions: Results from this new centralized ENIGMA-Epilepsy rsfMRI dataset support the hypothesis of transdiagnostic network segregation loss in epilepsy. Both FE and GE showed reduced within-network degree in associative networks, particularly default mode and salience systems, accompanied by increased between-network degree in sensorimotor and subcortical regions. This pattern suggests a shift from specialized within-network organization toward greater cross-network integration. In FE, abnormalities also carried lateralizing information, with stronger and more specific effects in TLE. These findings support a network-informed, ROI-level ENIGMA framework for mapping shared and syndrome-specific FC abnormalities. Future studies will link these FC signatures to clinical characteristics, treatment response, and surgical outcomes.

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Single Nucleus Transcriptomics Identifies an Epilepsy-Associated Microglial Population in the Pilocarpine Model of Temporal Lobe Epilepsy

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Background: Mesial temporal lobe epilepsy (TLE) is the most common form of acquired epilepsy involving the hippocampus and is a frequent sequelae of head trauma. TLE is associated with refractory seizures and significant cognitive deficits. Yet, the gene expression patterns and cell types driving epileptogenesis and the associated cognitive deficits are poorly understood.

Method: We performed single nucleus RNA sequencing on hippocampal tissue from mice at 3 and 6 weeks following pilocarpine-induced status epilepticus, a robust model of TLE.

Results: At these early timepoints, epilepsy samples showed reductions in specific Cck and Lamp5-Lhx6 interneuron subclusters, alongside increases in Cajal-Retzius cells, dentate granule (DG) cell precursors, and a mature DG cell subcluster. Among glia, an astrocyte subcluster and a markedly expanded microglia subcluster were increased. We term this microglia population epilepsy-associated microglia (EAM). The transcriptomic profile of EAM partially overlaps with microglia described in models of Alzheimer's disease and traumatic brain injury, both disorders which have increased risk of developing TLE. EAM display amoeboid morphology, can be found in dense clumps around pyramidal and granule cell body layers, and exhibit enlarged vesicles and mitochondria on electron microscopy. Cell-cell interaction analyses predict that DG cells are the main interaction partners of EAM. This dataset recapitulates known cellular alterations in TLE while defining their underlying transcriptomic programs, enabling mechanistic dissection of the key processes driving epileptogenesis.

Callosal Conduction Velocity Determines Bilateral Synchrony and Epilepsy Severity

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Abstract: Severe generalized epilepsy, as occurs in Scn8a loss of function, is defined by progressive seizures with simultaneous onset in the bilateral hemispheres (bilateral hypersynchrony), that increase in quantity over time. The physiological determinants of bilateral synchrony and epilepsy progression remain unclear. White matter tracts have traditionally been considered as fixed, passive conduits, including for transmission of seizure activity in epilepsy, but recently it has been proposed that white matter conduction is dynamic and may critically shape the timing of large-scale network interactions. We investigated conduction dynamics of major white matter tracts in the generalized seizure network, the corpus callosum and internal capsule, in Scn8a^{+/−} mice with generalized epilepsy. With slice electrophysiology, and stimulation of the ipsilateral corpus callosum, we recorded contralateral compound action potentials (CAPs) and found callosum conduction velocity to be pathologically increased. The increased conduction velocity was restricted to anterior callosum, a portion of the seizure network, occurred only in myelinated fibers and occurred only after the developmental onset of seizures. None of the other properties of callosal conduction we assessed (CAP amplitude, area, duration and rise time) differed between Scn8a^{+/−} and littermate controls. No differences were seen in any CAP conduction measure in the internal capsule. Given that myelination influences conduction velocity, we assessed network changes in myelination with MRI myelin mapping. This revealed increased myelination, selectively in the anterior corpus callosum, and overlapping the region of increased callosal conduction velocity. Genetic disruption of activity-regulated myelin plasticity, which we previously have shown to block seizure progression, prevented the increase in callosal conduction velocity, and reduced pathological bilateral hypersynchrony assessed with EEG coherence. Computational modeling of the generalized seizure network, informed by empirical measures in Scn8a^{+/−} mice, predicted that increased callosal conduction velocity would be sufficient to drive bilateral hypersynchrony and increase seizure severity. Consistent with these findings, focal anterior callosal chemical disruption of myelination with lysophosphatidylcholine in Scn8a^{+/−} mice decreased callosal conduction velocity without disrupting axonal transmission or inducing persistent neuroinflammation, and prevented epilepsy progression. Together, these results identify dynamic callosal conduction velocity as an unexpected determinant of bilateral hypersynchrony and of epilepsy severity.

Striatal Interneuron Dysfunction as a Novel Mechanism for Autism Related Features in Dravet Syndrome

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Background: Autism spectrum disorder (ASD) and epilepsy are highly co-occurring neurodevelopmental disorders however they have largely been studied separately, missing the opportunity to determine if there are shared underlying pathophysiological mechanisms. Dravet syndrome (DS) exemplifies this overlap as it presents with both infantile onset treatment-resistant epilepsy and high incidence of ASD. DS is caused by variants in SCN1A encoding the voltage-gated sodium channel subunit Nav1.1 which is prominently expressed in GABAergic inhibitory neurons (INs) throughout the brain. SCN1A is the most commonly identified gene in epilepsy and is also independently a validated ASD risk gene. DS (Scn1a^{+/−}) mice develop early-onset seizures and exhibit endophenotypes of ASD including hyperactivity, stereotyped movements, impaired learning and social behavior deficits. Hence,

there is a strong rationale for using this model to study basic mechanisms of ASD, which will further our understanding of the convergent and divergent pathophysiology of ASD and epilepsy. Previous work in Scn1a+/- mice have established deficits of INs in neocortex and hippocampus contributing to seizures. Whether changes in E/I balance within specific neural circuits underlie ASD symptoms vs seizures in DS remains an open question. A strong candidate is the striatum given known roles of striatal dysfunction in the motor stereotypies, behavioral inflexibility and altered social interactions characteristic of ASD. The two main subtypes of striatal GABAergic INs, parvalbumin (PV) and somatostatin (SST), also express Scn1a, but it is unknown how these INs are impacted in Scn1a+/- mice.

Methods: In the Scn1a+/- mouse model of DS we use a combination of acute brain slice electrophysiology and optogenetics to probe neuronal excitability and corticostriatal synaptic connections. Quantitative behavioral analysis is performed using Deep Lab Cut (DLC) tracking and Motion Sequencing (MoSeq) to assess spontaneous motor patterns and social interactions.

Results: First, we discover that both striatal SST and PV INs are hypoexcitable in Scn1a+/- mice, but with contrasting developmental time course. Striatal SST INs have early mild and transient hypoexcitability. PV INs uniquely have normal action potential firing at the time of seizure onset, then later develop severe and persistent deficits with inability to sustain fast action potential firing. We then use optogenetics to investigate the impact on excitation-inhibition balance in corticostriatal circuits, finding that feed-forward inhibition is largely intact due to increased excitatory drive onto PV INs in Scn1a+/- mice. MoSeq reveals altered motor pattern in Scn1a+/- mice across development, while hyperactivity only emerges in young adult mice. DLC tracking of multiple mice captures three types of social interactions. Scn1a+/- mice have reduced social interaction driven by a specific decrease in side by side huddling among Scn1a+/- dyads.

Conclusions: Our findings highlight striatal interneuron dysfunction as an underexplored locus of pathology in DS. The delayed and persistent impairment in striatal PV INs is compelling as this pattern aligns with the observation in mice and human patients of early severe epilepsy that improves over time while ASD features emerge and persist. High throughput machine learning based analysis of spontaneous behavior across development uncovers detailed quantitative motor and social phenotypes relevant to ASD. We propose a novel combination of intersectional genetic tools to probe the roles of PV and SST INs in seizures vs motor and social phenotypes. These findings will identify the key cell types and circuits involved in epilepsy vs ASD features in DS which informs how emerging genetic and cellular therapies for DS could be targeted to improve both epilepsy and developmental outcomes.

Quantitative EEG Biomarkers to Support Clinical Trial Readiness in SYNGAP1-Related Disorders

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Background: SYNGAP1-related disorders (SYNGAP1-RD) are rare neurodevelopmental conditions characterized by developmental impairment, epilepsy, and substantial clinical heterogeneity. As disease-modifying therapies advance toward clinical trials, there is an urgent need for objective, scalable biomarkers that can quantify disease severity, track disease trajectory, and detect treatment response. Quantitative EEG (qEEG) offers a promising approach by providing noninvasive, reproducible measures of brain function that can be collected longitudinally across clinical sites. This study aims to identify and validate qEEG biomarkers in SYNGAP1-RD that correlate with clinical severity and developmental outcomes, with the goal of supporting endpoint development for future disease-modifying therapeutic trials.

Methods: Resting-state EEG data were obtained from individuals with SYNGAP1-RD enrolled in the prospective ProMMiS natural history study, as well as from age-matched control participants. Recordings were processed using a standardized quantitative EEG workflow adapted from previously published methods (Galer, McKee et al., Neurology, 2025). The pipeline included artifact removal, selection of stable EEG epochs, and Welch-based spectral analysis to estimate regional power within canonical frequency bands. Relative power and band-power ratios were then computed across scalp regions. Random forest classifiers were trained to differentiate individuals with SYNGAP1-RD from controls, and model-derived feature importance was used to prioritize the spatial-spectral features most strongly contributing to classification as candidate biomarkers. Clinical relevance of EEG-derived features was assessed by examining associations with standardized developmental measures, including Bayley-4 and GMFM-66 scores, and seizure histories collected contemporaneously with the EEG recordings.

Results: Random forest models demonstrated robust performance in distinguishing EEGs from individuals with SYNGAP1-RD from controls (AUC 0.86), and occipital alpha/theta ratios emerged as the most informative feature by Gini importance. Furthermore, several spectral EEG features including the occipital alpha/theta ratio demonstrated significant correlations with standardized developmental outcome measures, such as the GMFM-66 ($\rho=0.42$, $p=0.04$), supporting their use as quantitative markers of disease severity. While seizures are abundant in SYNGAP1-RD, there were no correlations identified between seizure frequency and occipital alpha/theta ratio, with $p>0.05$ for frequency of myoclonic, atonic, and absence seizures, as well as the overall seizure frequency score.

Conclusions: qEEG-derived spectral features prioritized through a machine learning-based pipeline represent reproducible, spatially specific biomarkers in SYNGAP1-RD. Reduced occipital alpha/theta in SYNGAP1-RD represents a disorder-specific signature that correlates with developmental outcomes. Furthermore, these qEEG spectral features are independent of seizure frequency scores, suggesting they may represent surrogate developmental markers not confounded by epilepsy severity. These findings support qEEG biomarkers as objective, scalable clinical trial endpoints for tracking disease progression and treatment response in SYNGAP1-RD.

Semi-Self-Supervised Representation Learning of Spike Waveforms Reveals Emergent Excitatory-Inhibitory Organization

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Background: Distinguishing excitatory from inhibitory neuronal activity on microelectrode local field potential recordings is important for understanding seizure mechanisms and cognitive processing. Current approaches require manual spike sorting followed by post-hoc classification using waveform features, limiting scalability. We aimed to develop a self-supervised framework that learns rich spike waveform representations without explicit cell-type labels, and to evaluate whether biologically meaningful structure — including excitatory/inhibitory identity — emerges from the learned embeddings.

Methods: We used a manually curated waveform dataset recorded from multichannel silicone probe recordings from 5 PV-Cre32 optogenetically tagged mice from the Buzsaki open access Datashare. When presented with an optogenetic pulse, parvalbumin-positive inhibitory interneurons are silenced, allowing for ground-truth classification of units. We further tested the method on the raw recording of a sixth mouse without manually sorted units. We extended the Barlow Twins self-supervised framework with unit-aware positive pair sampling: during training, two spikes from the same neuron are independently augmented with realistic perturbations (noise injection, amplitude scaling, baseline shifts) and encoded by a deep fully connected network. The loss function drives same-unit embeddings toward agreement while decorrelating embedding dimensions to prevent collapse. No cell-type labels are provided during training. We evaluated generalization using leave-one-mouse-out cross-validation and assessed emergent structure via UMAP visualization and cosine similarity analysis.

Results: We used ~2.67 million waveforms arising from the 5 mice. The learned representations exhibited clear emergent organization in held-out mice unseen during training. Spikes from individual neurons formed tight clusters in embedding space, and excitatory and inhibitory populations separated into distinct regions despite the absence of cell-type supervision. The method showed excellent excitatory/inhibitory waveform classification in leave-one-mouse-out testing (ROC-AUC range 0.9-0.99; average 0.95). Cosine similarity matrices sorted by cell type confirmed high within-class similarity and between-class differentiation. In the sixth mouse, our method accurately detected the dropout of inhibitory firing and reciprocal increase in excitatory firing during the optogenetic stimulations.

Conclusions: Self-supervised learning on spike waveforms discovers cell-type identity without manual labels, providing a scalable foundation for downstream analyses in epilepsy and cognitive neuroscience research. Our approach extends the Barlow Twins redundancy-reduction framework from image processing with a novel unit-aware positive pair sampling.

Frequency-Specific Thalamocortical Network Dynamics During Focal Seizures in Humans

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Background: The thalamus is increasingly targeted for neuromodulation in drug-resistant epilepsy, including responsive neurostimulation and stimulation strategies. However, the temporal, frequency-specific, and directional features of thalamocortical involvement during human focal seizures remain incompletely defined. We sought to characterize thalamocortical network dynamics across seizure evolution, compare them with cortico-cortical propagation, and determine whether thalamic signals contain seizure-state information relevant to responsive and adaptive thalamic neuromodulation.

Methods: We retrospectively analyzed stereo-electroencephalography (SEEG) recordings from 19 patients with pediatric-onset drug-resistant focal epilepsy who underwent simultaneous cortical and thalamic sampling, comprising 66 focal seizures. Thalamic electrodes sampled the anterior nucleus (AN) and/or centromedian nucleus (CM). SEEG signals were analyzed in bipolar montage across preictal, early ictal, end-of-seizure, and postictal epochs relative to interictal baseline. Thalamic ictal patterns were visually classified by epileptologists. Spectral power, imaginary coherence, and spectral Granger causality were computed in slow (1–12 Hz), beta (13–30 Hz), and gamma (30–70 Hz) bands to quantify local thalamic activity, thalamocortical synchronization, and directed interactions between thalamic nuclei and the seizure onset zone (SOZ). Cortico-cortical interactions were analyzed for comparison. Random forest classifiers using temporally resolved thalamic features were trained separately for AN and CM to distinguish ictal from non-ictal states using leave-one-patient-out cross-validation; feature contributions were assessed using ablation analyses and SHAP values.

Results: The cohort included 1,599 SEEG contacts, including 101 thalamic contacts. Thalamic ictal activity was present at seizure onset in most sampled contacts and became nearly universal by seizure termination. Visual patterns evolved from low-voltage fast activity at onset toward rhythmic spike/spike-wave or rhythmic slow-wave activity at termination. Both the thalamus and SOZ showed early broadband spectral power increases, followed by attenuation after seizure termination. Slow- and beta-band thalamic

power were especially prominent near seizure termination. Thalamocortical imaginary coherence increased during seizures, most consistently in slow and beta bands during ictal and end-of-seizure periods. Directed connectivity analyses demonstrated bidirectional thalamocortical interactions dominated by slow and beta frequencies. Slow-frequency outflow from both AN and CM toward the SOZ exceeded propagation-zone-to-SOZ cortico-cortical outflow during ictal and end-of-seizure periods, suggesting that late ictal thalamocortical engagement was not simply a passive reflection of cortical spread. Exploratory subgroup analyses suggested stronger AN–SOZ connectivity in limbic seizures and greater slow-band connectivity in seizures with rhythmic spike/spike-wave or rhythmic slow-wave onset patterns. Thalamic features classified seizure state with mean AUROC values of 0.825 ± 0.163 for AN and 0.839 ± 0.149 for CM. Performance improved when thalamocortical connectivity and directed-connectivity features were added to spectral power alone. Key predictors included thalamic slow/beta power, slow-frequency thalamus-to-SOZ outflow, and beta-frequency SOZ-to-thalamus inflow.

Conclusions: Human focal seizures engage dynamic, frequency-specific, and direction-specific thalamocortical interactions that evolve from seizure onset to termination. The prominence of slow- and beta-band bidirectional thalamocortical coupling, particularly during late ictal stages, suggests that thalamic involvement is a structured component of ictal network evolution and is not fully captured by cortico-cortical propagation alone. Together, the temporally resolved classification of seizure state from thalamic features and the identification of frequency- and direction-specific thalamocortical signatures support the potential utility of thalamic biomarkers for responsive and adaptive neuromodulation strategies that detect seizures, track evolving ictal network states, and trigger or adjust stimulation accordingly. Prospective studies are warranted to test whether these thalamocortical biomarkers can improve seizure detection and individualized thalamic stimulation strategies in drug-resistant epilepsy.

Optimizing Sleep Architecture and Cognitive Function in New-Onset Temporal Lobe Epilepsy

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Background: Accumulating evidence indicates that individuals with new-onset temporal lobe epilepsy (TLE) frequently exhibit comorbid sleep disturbances and cognitive dysfunction. Prior studies suggest that acoustic stimulation (AS) during sleep may enhance sleep architecture and improve cognitive performance. However, the presence and clinical significance of sleep architecture abnormalities in recent-onset TLE remain unclear. This study examined sleep architecture and its association with cognitive functioning in young adults with new-onset TLE.

Methods: Thirty youth with new-onset TLE (aged 18-25 years) were compared with 30 age- and sex-matched healthy controls. Participants underwent overnight polysomnography using standard 10–20 PSG monitoring, one night with AS and one night without AS. Sleep spindle density, slow-wave oscillations, and spindle–slow-wave coupling were evaluated. In addition, participants completed comprehensive neuropsychological testing assessing intelligence, language, immediate & delayed verbal memory, executive function, and processing speed. To determine clinical predictors, clinical epilepsy characteristics (age of onset, seizure syndrome, seizure frequency and antiseizure medication), and magnetic resonance imaging (MRI) and electroencephalography (EEG) abnormalities were also collected.

Results: Compared with controls, individuals with TLE demonstrated reduced sleep spindle density, diminished slow-wave oscillations, and weaker spindle–slow-wave coupling, which improved with acoustic stimulation. Immediate and delayed verbal memory performance also improved post-stimulation TLE group. Clinical predictors indicated that earlier age of epilepsy onset was associated with greater alterations in sleep architecture. In addition, higher rates of interictal epileptiform discharges were associated with more severe sleep-related abnormalities.

Conclusions: These findings demonstrate that alterations in sleep architecture are present early in the course of TLE and are associated with cognitive dysfunction. The results further suggest that sleep-based interventions targeting spindle and slow-wave activity may represent a promising avenue for improving cognitive outcomes in this population. Future studies should evaluate the long-term therapeutic potential of optimizing sleep architecture in new-onset TLE.

Microbleed Burden Predicts Cognitive Impairment in Older Adults with Epilepsy

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Background: Older adults with epilepsy are at elevated risk for cognitive impairment and dementia, yet the biological mechanisms underlying this vulnerability remain incompletely understood. Although Alzheimer's disease pathology has been increasingly investigated in epilepsy, cerebrovascular small vessel disease (SVD) represents another potential contributor to cognitive decline. Emerging evidence suggests a complex relationship between epilepsy and cerebrovascular injury, whereby vascular pathology may promote seizures and seizure-related processes may further exacerbate vascular damage. However, the extent to which specific MRI markers of SVD

contribute to cognitive dysfunction beyond normal aging, and whether cerebrovascular profiles differ between early- and late-onset epilepsy, remains unclear.

Methods: Ninety older adults with epilepsy (mean age 66.1 ± 6.8 years, 57% female; 52 early onset <55 years, 38 late onset ≥ 55 years) underwent 3T MRI including quantification of white matter hyperintensities (WMH; deep and periventricular), perivascular spaces (PVS; basal ganglia and whole brain), and cerebral microbleeds. Cognitive assessment included the MoCA and an 8-test neuropsychological composite. Participants from ADNI (CN=339, MCI=303, AD=83) served as comparison groups for cerebrovascular burden and cognitive analyses. Principal components analysis (PCA) was used to characterize the latent structure of SVD markers.

Results: Participants with early-onset epilepsy demonstrated greater periventricular WMH burden than age-matched cognitively normal controls ($p < .01$), whereas late-onset epilepsy did not differ from controls. Basal ganglia PVS burden was lower in epilepsy than CN participants ($p < .05$), an effect more pronounced in the early-onset group. Microbleeds were observed in 27% of epilepsy participants, approximately double the prevalence observed in cognitively normal older adults (14%; OR=2.6, 95% CI 1.2–5.6, $p < .05$) and higher than in MCI participants (17%; OR=3.4, 95% CI 1.5–7.8, $p < .01$), despite comparable vascular risk burden. Microbleeds were the only SVD marker associated with cognition, demonstrating a dose-dependent relationship with MoCA performance (0 microbleeds=25.2, 1=24.5, ≥ 2 =21.6; $p < .01$). This relationship was evident in both epilepsy groups but stronger among individuals with late-onset epilepsy. Comparable associations were not observed in ADNI CN or MCI participants. PCA identified a microbleed-related dimension independent of WMH/PVS burden (22.9% variance explained) that significantly predicted MoCA performance ($p < .05$).

Conclusions: Older adults with epilepsy exhibit a heterogeneous cerebrovascular profile in which conventional markers of SVD and cerebral microbleeds appear to carry distinct clinical significance. While elevated periventricular WMH burden characterized early-onset epilepsy, microbleeds emerged as the dominant cerebrovascular correlate of cognitive impairment and occurred at disproportionately high rates relative to aging comparison groups. The absence of similar microbleed-cognition associations in cognitively normal and MCI older adults suggests that epilepsy may amplify the cognitive impact of cerebrovascular injury. These findings identify microbleed burden as a promising marker of cognitive vulnerability in epilepsy and highlight potential contributions of vascular mechanisms, including blood-brain barrier dysfunction and cerebral amyloid angiopathy, to late-life cognitive outcomes.

Profiling and Engineering the Ion Channel Transcriptome

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Background: In the human brain, a large repertoire of ion channels regulates the excitability of neurons, circuits, and networks, generating complex human cognition and behavior. There are over 200 genes encoding various ligand- and voltage-gated ion channels as well as auxiliary proteins. This vast repertoire is further amplified by alternative splicing, which generates up to hundreds of isoforms for each gene. During brain development, many ion channels undergo changes in splicing that critically regulate neuronal function. Dysregulated splicing of ion channels is implicated in disorders ranging from epilepsy to neurodegenerative diseases. While previous studies have highlighted the roles of individual ion channel splicing events in brain development, homeostasis, and disease, there has never been a systematic study of the alternative splicing of ion channels. Two major barriers have impeded a systematic study of ion channel isoforms. First, most ion channels are encoded by large, multi-exon genes with complex alternative splicing. Second, ion channels are orders of magnitude lower in abundance compared to housekeeping genes. One recent study showed that these barriers can be overcome by combining two technologies: 1) long-read RNA-sequencing, which generates full-length transcripts over 5-10 kb in length, and 2) transcript capture, which can enrich for lowly expressed transcripts by up to several hundred-fold. This approach has been validated in studies of alternative splicing in cancer, as well as for several brain-enriched genes, but it has not been applied to study ion channels. As such, the repertoire of ion channel isoforms in the brain is likely vastly underappreciated. My own research showed that SCN1A, a gene encoding a subunit of the voltage-gated sodium channel, utilizes alternative poison exon splicing during human brain development to regulate transcript abundance. Furthermore, aberrant splicing of these poison exons occurs in certain individuals with Dravet syndrome, and RNA therapeutics can rescue protein production, neuronal excitability, and disease phenotypes. Here, I follow the example of SCN1A to develop a systematic approach to study the alternative splicing of ion channels (Aim 1), with the hypothesis that there are numerous unidentified isoforms of ion channels which are differentially regulated during human brain development. Additionally, I develop a system to evaluate the functional impact of alternative splicing, using the human BK channel as a proof of concept, to demonstrate that developmentally regulated isoforms of this gene differentially impact the excitability of human stem cell-derived neurons (Aim 2).

Methods: In Aim 1, I combine transcript capture and long-read sequencing to systematically identify the isoforms of 226 voltage- and ligand-gated ion channel genes in the cerebral cortex of the neonate and young adult. Using existing and new bioinformatic tools, I will 1) generate an expanded list of ion channel isoforms with annotations of predicted protein-coding capacity, 2) estimate their relative abundance for each gene, and 3) identify isoforms that are differentially expressed between the brains of neonates and young adults. The result will be the most comprehensive resource of ion channel isoforms to-date, providing the basis for additional studies to evaluate the functional impact of ion channel splicing. In Aim 2, using a splice modulation toolkit, I will interrogate the functional impact of alternative splicing in the BK channel (encoded by KCNNM1) in human stem cell-derived neurons, comparing the conditions of total knockdown, single exon-switching, and isoform-switching. Patch-clamp electrophysiology will be used as the primary readout to study the consequences of splice modulation on action potential kinetics, afterhyperpolarization, and firing frequency. The results will provide

an isoform-specific level of understanding of KCNMA1 function and identify novel RNA therapeutic targets for BK channelopathies.

Results: In a pilot experiment, I combine transcript capture and long-read RNA-sequencing to study 42 epilepsy-associated ion channel genes in two samples of human cerebral cortex from neurotypical individuals at two developmental timepoints, neonate (1 month old) and adult (age 27). I demonstrate that this approach achieves an average of 70-fold enrichment for the 42 targeted ion channels, leading to a 50-60% on-target rate. These results demonstrate that this approach is highly effective for increasing the depth of coverage for ion channel genes. Taking KCNMA1 as an example, I show distinct patterns of developmental regulation of two functionally important, alternatively spliced cassette exons in this gene. Adopting an existing splice-modulation toolkit, I demonstrate the feasibility of splice-switching exons at endogenous loci in a heterologous cell line.

Conclusions: Here, I demonstrate proof-of-concept for combining transcript capture and long-read RNA-sequencing to achieve deep coverage of the ion channel transcriptome in the human brain. Next, I will use this approach to generate an atlas of developmentally regulated transcript isoforms for the approximately 226 human ion channel genes. I will also create a readily accessible web application to enable easy visualization. Additionally, I will generate a splice modulation toolkit in stem cell-derived neurons to study the functional impact of alternative splicing events in ion channels, including the human BK channel. Taken together, the skills and resources developed in this project serve as a platform for a multi-modal investigation of the role of alternative splicing in disease contexts. My future aims are to 1) identify pathologic splicing patterns in epilepsy using disease models and surgically resected human brain tissue, and 2) use splice modulation to discover RNA-targeting therapeutics for epilepsy and other disorders of network excitability.

Parvalbumin-positive Interneuron Density and Intrinsic Property Alterations in a Mouse Model of Pcdh19 Clustering Epilepsy

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BACKGROUND: Protocadherin-19 (PCDH19)-clustering epilepsy (PCE) is a severe developmental and epileptic encephalopathy (DEE) and one of the most common monogenic epilepsies. It is characterized by cognitive impairment and intractable seizure clusters with onset in the first few years of life. PCDH19 is an X-linked gene that encodes a transmembrane cell adhesion molecule critical for cell-cell interactions during brain development. PCE affects females and rare mosaic males, while hemizygous males expressing only mutant PCDH19 do not develop epilepsy. A leading hypothesis to explain this phenomenon is that it occurs due to cellular interference associated with random X-inactivation (or mosaic mutations) in which cells expressing only wild type and those expressing only mutant PCDH19 fail to interact properly during brain development. This is demonstrated in our mouse model in which we see a striking pattern in which the Pcdh19+ and null neurons (after random X-inactivation) segregate from one another in the cortex, hippocampal CA1 region, and in interneuron progenitors in the ganglionic eminences. PCE mice show a lower seizure-threshold and more severe seizures when exposed to hyperthermia. Given the key role of inhibitory interneurons in many genetic epilepsies, we sought to examine parvalbumin (PV) interneuron distribution and intrinsic properties based on regional Pcdh19 expression.

METHODS: Female Pcdh19+/-; PV-cre mice were crossed with X-GFP; tdTomato cre-reporter males. Female offspring expressed GFP on approximately half of their neurons, while males do not express GFP. All PV-cre x tdTomato offspring expressed tdTomato on PV-positive interneurons. Patch clamp electrophysiology was performed on acute slices in the cortex at P25-32. Histological studies were performed on fixed brain sections at P45-90.

RESULTS: PV-interneuron density was decreased in the CA1 region of the hippocampus in PCE mice compared to WT controls. When examining the cortex, there was no difference in overall cell density between groups. However, PV-density was significantly lower in the GFP-/Pcdh19- regions compared to GFP+/Pcdh19+ regions of the cortex. Current clamp electrophysiology revealed hypoexcitability of both the PV and non-PV neurons in the knockout males when compared to wild-type. In PCE females, PV+ neurons within the GFP+ regions displayed similar firing patterns to WT mice. However, in the GFP- regions, there was a leftward shift in the firing pattern of the PV+ interneurons, with early depolarization block that was not observed in the GFP+ regions.

CONCLUSIONS: PV-interneuron density is decreased in the hippocampus and Pcdh19- regions of the cortex of PCE mice. Intrinsic properties of PV-interneurons are altered in the Pcdh19- regions of the cortex such that they fire more with physiologic current input, but reach early depolarization block with high current input – a phenomenon observed in other DEE mouse models. This pattern was not observed in the knockout mouse, suggesting that it is not simply due to cell genotype and may instead be related to transcriptional changes in cellular properties that are altered in the mosaic state. This suggests that seizures in PCE may be driven by abnormal PV-tone in the mutant regions of a Pcdh19-mosaic brain. Further examination of circuit dynamics will provide further insight to the underlying mechanisms of PCE.

Global Neurology

Predictors of Caregiver Burden and Depression Among Zambian Caregivers of Older Adults with Neurologic Disease

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Background: With aging populations, long term care needs have dramatically increased in communal societies like Zambia and fall largely on family members as informal caregivers. While caregiver burden has been associated with greater depression in Western settings, little is known about the impact of these demands on caregiver outcomes in Zambia, particularly when providing care to older adults with neurologic disease. This study aimed to evaluate caregiver burden and depression and their predictors and investigate whether burden and depression differ among Zambian caregivers based on participant medical history.

Methods: The Zambian Cohort of Healthy Aging and Dementia is a population-based cross-sectional study of aging of adults ≥ 55 years and their informal caregivers. Patient and caregiver sociodemographic characteristics, caregiver burden, and depression were assessed. Participants underwent a complete medical history, physical examination, and neuropsychologic testing to assess neurologic diagnoses. Caregiver burden was assessed using the Zarit Burden Inventory (high burden score >20) and depression via the PHQ-9 (depression score >10). Multivariable logistic regression assessed associations between participant and caregiver characteristics, participant medical history, and caregiver burden and depression.

Results: To date, 277 participant (mean age 66 years, 75% female) and caregiver pairs have completed data collection. Participant medical history included HIV (21%), diabetes (11%), stroke (11%), and epilepsy (5.4%). 56% reported a history of hypertension, of whom 24% were in hypertensive emergency during study participation. 15% of participants had dementia.

Caregivers (mean age 34 years; 75% female, mean monthly income 80 USD) were most frequently the participant's child (58%) or grandchild (21%). 44% had additional family/friends to aid with caregiving tasks, but only 8% had paid help and 22% had given up or cut back on work due to caregiving. 54/277 (19%) caregivers reported ongoing caregiver burden. Burden was associated with household wealth, with 54% of those experiencing caregiver burden in the lowest household wealth quartile compared to 23% of those not experiencing burden ($p < 0.01$). The availability of additional family members to aid with caregiving tasks did not decrease burden. After adjusting for caregiver sex, age, household wealth, and the presence of other caregivers, caring for a participant with dementia (OR 3.63 [95% CI 1.69-7.84]) or hypertension (OR 2.16 [95% CI 1.12-4.27]) was associated with greater burden. Participant history of HIV, stroke, epilepsy, or diabetes did not significantly impact burden. 38/277 (14%) caregivers met criteria for moderate to severe depression, which was associated with increased caregiver burden (OR 3.88 [95% CI 1.75-8.56]). There were no other significant predictors of depression.

Conclusions: Informal caregiving is associated with significant burden among caregivers in Zambia, which in turn, leads to greater likelihood of depression. Health outcomes of informal caregivers have been largely overlooked in this setting and improving formal care for conditions like hypertension and dementia may indirectly improve caregiver wellbeing in addition to patient outcomes.

Headache and Pain

Clinical and Network Predictors of Response to Motor Cortex repetitive Transcranial Magnetic Stimulation (rTMS) for Trigeminal Neuropathic Pain: A Randomized Controlled Trial

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Background: Trigeminal neuropathic pain (TNP) is notoriously severe, debilitating, and often refractory to conventional treatments. Repetitive transcranial magnetic stimulation (rTMS) is a promising non-invasive treatment modality that uses focused magnetic pulses to modulate brain activity. Although rTMS is FDA approved for depression and obsessive-compulsive disorder and is widely used in Europe for neuropathic pain, its role in pain management remains experimental in the United States. Prior studies indicate that high frequency (≥ 10 Hz) rTMS (HF-rTMS) targeting the primary motor cortex (M1) contralateral to pain is effective for neuropathic pain, but treatment responses are variable and analgesic mechanisms remain poorly understood. To evaluate the efficacy of HF-rTMS and identify predictors of treatment response, we conducted a pilot randomized controlled trial comparing HF-rTMS to active-control low-frequency (1 Hz) rTMS (LF-rTMS) in patients with TNP.

Methods: We conducted a randomized parallel-group trial in adults aged 18–80 years with unilateral neuropathic facial pain. Single-pulse TMS was used to identify the abductor pollicis brevis motor hotspot contralateral to pain and determine resting motor threshold (RMT). Neuronavigation software was used to identify and register the individualized M1 treatment target for precise stimulation and extraction of neural data. Participants were randomized 1:1 to HF-rTMS (10 Hz, 3,000 pulses/session, 30,000 pulses total) or LF-rTMS (1 Hz, 300 pulses/session, 3,000 pulses total) delivered at 90% RMT for 10 consecutive weekdays. Participants underwent magnetic resonance imaging (MRI) before treatment and after the final stimulation session, including structural imaging and resting-state functional MRI (rs-fMRI). The primary endpoint was change in visual analog scale (VAS) pain intensity after 10 treatment days. Secondary endpoints included validated measures of pain, mood, disability, and sleep, collected through 6-month follow-up. Group differences were evaluated using constrained longitudinal mixed-effects models. Functional connectivity and graph-theoretical measures were derived from preprocessed and denoised rs-fMRI data using subject-specific whole-brain network parcellations. Electric-field modeling was performed using SimNIBS to identify the stimulated M1 target for

each participant.

Results: Thirty participants completed all study procedures (HF-rTMS n=16; LF-rTMS n=14). Groups were comparable at baseline with respect to demographic, clinical, and TMS-related variables. Blinding integrity was preserved, with 54.5% of participants receiving HF-rTMS and 75.0% receiving LF-rTMS believing they had received active stimulation. HF-rTMS produced significantly greater reductions in pain intensity than LF-rTMS at the primary endpoint after 10 treatment days (mean difference = -1.57/10 VAS points, $t=-2.66$, $p=0.008$). Significant between-group differences were also observed at 1-week follow-up (mean difference = -2.29/10, $t=-3.93$, $p<0.001$) and 1-month follow-up (mean difference = -1.65/10, $t=-2.50$, $p=0.012$). At 1 week, 50% of HF-rTMS participants achieved responder status ($\geq 30\%$ pain reduction) compared with 8% of LF-rTMS participants, while 38% of HF-rTMS participants achieved very meaningful relief ($>50\%$ pain reduction) compared with 0% of LF-rTMS participants. Similar trends were observed for pain disability measures. Adverse effects were mild and included transient scalp discomfort, fatigue, and lightheadedness. Multiple regression analyses identified several predictors of treatment response. A significant interaction between stimulation intensity and treatment group indicated that higher stimulation intensities predicted greater pain relief following HF-rTMS but not LF-rTMS ($\beta=4.18$, $t=3.00$, $p=0.007$). Current gabapentinoid use was associated with reduced treatment benefit, with a significant frequency-by-gabapentinoid interaction demonstrating lower likelihood of response among patients taking gabapentin or pregabalin ($\beta=-54$, $t=-2.20$, $p=0.038$). Baseline graph-theoretical analyses revealed a significant interaction between M1 node strength and treatment group ($\beta=0.83$, $t=2.60$, $p=0.017$), such that HF-rTMS targeting highly connected M1 regions predicted greater improvement whereas LF-rTMS targeting highly connected M1 regions predicted worse outcomes. Incorporating M1 node strength into a multivariate model containing age, sex, baseline pain, stimulation intensity, and gabapentinoid use increased explained variance in pain relief from 49% to 62% ($\Delta R^2=13\%$, $p=0.049$).

Conclusions: Ten days of HF-rTMS targeting M1 was safe, well tolerated, and produced clinically meaningful reductions in pain relative to active-control LF-rTMS in patients with trigeminal neuropathic pain. Both treatment-related factors (stimulation intensity, medication use) and network-level characteristics of the stimulated M1 target predicted clinical outcomes. These findings support a network-based mechanism of analgesia and suggest that individualized targeting strategies based on brain connectivity, perhaps combined with optimized TMS dosing and concomitant medication strategies, may improve the efficacy of rTMS for neuropathic pain. Ongoing analyses are evaluating longitudinal effects of rTMS on whole-brain network organization and their relationship to clinical outcomes.

Health Services and Health Equity

A Trauma-Informed Cognitive Rehabilitation Program for Refugees with Traumatic Brain Injury and Cognitive Impairment: An Open Pilot Study

Presenting Author: *Altaf Saadi, MD, MSc, Massachusetts General Hospital, Harvard Medical School*

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Objectives: Traumatic brain injury (TBI) is more prevalent among refugees and asylum-seekers (hereafter, refugees), yet no existing interventions are tailored to their needs or to other similarly trauma-exposed populations. We adapted a cognitive rehabilitation program (“EducaBrain”) delivered in English and Spanish in a trauma-informed, culturally tailored manner via live video, using paraprofessional delivery, to engage refugees with TBI and perceived cognitive impairment.

Methods: Participants were adults aged 18–65 (to limit age-related confounding), English or Spanish speaking, self-identified refugees, with mild or moderate traumatic brain injury (TBI) sustained in adulthood (>18 years) using the Ohio State University TBI Identification (OSU TBI-ID), and reporting subjective cognitive impairment on either the PROMIS Cognitive Function score or the cognitive items of the Neurobehavioral Symptom Inventory (NSI). We conducted a feasibility open pilot with pre- and post-intervention assessments, along with qualitative exit interviews, to evaluate the feasibility and preliminary outcomes of the adapted cognitive rehabilitation program. We iteratively adapted the program throughout the open pilot process.

Results: The sample included 5 English-speaking and 5 Spanish-speaking refugees ($n=10$), with a mean age of 39.7 years (SD 6.7). Half were women, and 60% had a high school education or less. Participants had lived in the U.S. for an average of 9.0 years (SD 3.7) and represented seven countries, most commonly Guatemala ($n=3$) and Uganda ($n=2$). There were pre-post improvements in PROMIS cognitive function and NSI cognitive domain scores, as well as reductions in NSI total scores, PHQ-9 scores, GAD-7 scores, and positive PTSD screens. Participants also reported high feasibility, acceptability, and satisfaction on the Feasibility of Intervention Measure (FIM), Acceptability of Intervention Measure (AIM), and Client Satisfaction Questionnaire (CSQ), respectively. We refined the program by shortening it to eight weeks, revising the manual to a 6th-grade reading level, adding positive psychology components in response to participant needs (e.g., self-compassion exercises), and incorporating content on flexible approaches to TBI recovery and self-advocacy skills. Preliminary thematic analysis of exit interviews identified three themes: (1) motivation for participation, driven by intrinsic and extrinsic factors including lived experience, curiosity, and advocacy; (2) perceived benefits, including cognitive, emotional, and personal growth outcomes and improved sense of self; and (3) barriers to participation, including time constraints, financial stress, emotional vulnerability, and technical challenges with the virtual format.

Conclusion: This trauma-informed, culturally adapted cognitive rehabilitation program for English- and Spanish-speaking

refugees, delivered via live video via paraprofessionals, was feasible and showed promise for improving cognitive and affective functioning. These findings inform a subsequent feasibility RCT of “EducaBrain” in preparation for efficacy testing.

Movement Disorders

Gait patterns in Progressive Supranuclear Palsy

Presenting Author: *Farwa Ali, MBBS, Mayo Clinic*

Background: Progressive Supranuclear Palsy (PSP) is a complex neurodegenerative disorder characterized by prominent motor, balance, and cognitive impairments. Identifying objective biomechanical markers that distinguish between various clinical phenotypes such as Richardson Syndrome, Cortical, and Subcortical variants is crucial for accurately tracking disease progression and severity. This study, encompassing 58 unique subjects with PSP, aims to quantify gait and balance characteristics using dimensional reduction techniques and to determine how these objective metrics correlate with established clinical severity scores. The cohort consisted of 55.2 % males and 44.8 % females with a mean age of 69.8 years and an average BMI of 27.6. Richardson Syndrome was the predominant subtype, representing 46.6 % of the participants.

Methods: Participants underwent comprehensive marker-based 3D level walking and balance testing to capture a robust set of biomechanical data. From these assessments, 35 relevant gait variables were extracted, incorporating both the mean and standard deviation for temporal distance parameters to account for gait variability. To analyze the high-dimensional data, Z-score normalization was applied to all variables before performing a Principal Component Analysis (PCA) configured for eight dimensions. To interpret the functional meaning of the resulting components, PCA loadings were plotted against the original variables. Independent T-tests were subsequently utilized to evaluate differences in these principal components across the distinct PSP clinical subtypes. Finally, correlation analyses were conducted between each PCA component and key clinical metrics, including MOCA cognitive scores (cohort average: 21.8), UPDRS-III motor scores (average: 41.9), PSP falls, overall PSP Rating Scale (PSPRS) totals (average: 34.7), and the PSPRS Gait Midline (PSPRS-GM) subscore. All statistical procedures were executed in Python using the SciPy and Statsmodels libraries.

Results: Preliminary comparisons of the 35 raw gait variables across PSP subtypes revealed that the standard deviation of step length differed significantly between the Richardson Syndrome and Subcortical groups, as well as between the Subcortical and 'others' groups. The subsequent PCA successfully distilled the data, with the eight extracted components accounting for 78 percent of the total variance in the dataset. Analysis of the component loadings revealed that PC1 and PC2 were heavily correlated with the majority of the overall gait variables. PC3 captured visually dependent postural stability, strongly correlating with the Romberg Ratio of both the Center of Mass and Center of Pressure. PC4, which was significantly tied to temporal distance parameters, emerged as the sole principal component demonstrating statistically significant differences across the different PSP subtypes. Further components captured other specific domains: PC5 represented dynamic stability, PC6 correlated heavily with raw cadence and velocity, and PC7 captured the variance (standard deviation) of that cadence and velocity. In terms of clinical relevance, PC1 exhibited significant correlations with nearly all clinical variables, functioning as an objective proxy for overall disease severity. Notably, PC1 demonstrated a highly significant inverse relationship with the PSPRS Gait Midline score ($p = 0.0000$).

Discussion: The application of Principal Component Analysis to level walking and balance data provides a robust, data-driven framework for characterizing gait impairments in Progressive Supranuclear Palsy. By reducing 35 disparate gait variables into eight functional components, the study effectively isolated distinct domains of motor dysfunction, ranging from temporal distance parameters to dynamic stability and visually dependent balance. The strong correlation between PC1 and standard clinical assessments underscores the validity of using objective biomechanical metrics to estimate overall disease severity, particularly concerning midline motor deficits. Furthermore, while the general gait profiles share significant variance across the cohort, specific metrics like step length variability and PC4 highlight measurable biomechanical distinctions between PSP clinical subtypes. These findings suggest that quantitative gait and balance analysis can serve as a highly sensitive, objective biomarker for evaluating both disease severity and phenotypic differences in PSP populations.

Video-based Quantitative Gait Analysis in Early Walkers

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Background: Laboratory-based pediatric gait analysis in neurology enables insights into underlying pathophysiology, guides surveillance, and informs treatment decision-making, but is not accessible to most U.S. children. We propose a video-based method of pediatric gait analysis drawing upon advances in computer vision to extract key kinematic features in a cohort of neurotypical young walkers. Our long-term goal is to use video-based approaches to gait analysis to improve early identification, monitoring, and treatment planning of gross motor impairment in pediatric neurology.

Methods: We conducted a retrospective observational study of toddlers walking on a straight path. We applied markerless pose estimation onto videos using OpenPose, an open-source machine learning model. We adapted an existing MATLAB pipeline to generate kinematic features such as step length, gait speed, and step time from key body features. Linear mixed model analyses were conducted in R on gait parameters to evaluate for effects of age (in months) and sex, accounting for multiple trials per participant.

Results: We analyzed sagittal videos of 83 neurotypical young walkers (42 females, 41 males, 13 to 19 months old). Participants completed 5.72 trials on average. Males had an increased step length ($\beta = 1.461$, $p = 9.28 \times 10^{-5}$) and gait speed ($\beta = 0.08$, $p = 6.75 \times 10^{-4}$) compared to females, but no main effect of sex was seen for step time. No statistically significant main effect of age was seen on any of the gait parameters.

Conclusions: Demonstrate that kinematic gait features are readily measurable from video and capture biologically meaningful differences in gait in terms of sex. The restricted age range of the participants concentrated around 14-15 months limits the conclusions that may be drawn on capturing developmental changes in video-based gait parameters over time. Our ongoing work will assess the validity of video-based gait features with synchronously collected data from a pressure-sensitive mat and prospectively evaluate videos of children with neurogenetic disorders compared to clinical scales.

Mechanistic Cross-talk Between LRRK2 and GBA1 in Regulating Lysosomal Function and Inflammatory Activity in Microglia **Presenting Author: Sarah Brooker, MD, PhD, Northwestern University Feinberg School of Medicine**

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Background: Examination of genetic etiologies of Parkinson's disease has revealed multiple genes involved in regulation of lysosomal function. This includes the two genes most commonly linked to Parkinson's disease, GBA1 and LRRK2. GBA1 encodes the lysosomal enzyme GCase and LRRK2 has been shown to regulate lysosomal function by multiple mechanisms. The goal of this study is to evaluate the mechanistic interaction between LRRK2 and GBA1 in regulating lysosomal function in microglia and to examine the impact of these genes on inflammatory responses of microglia to CNS substrates.

Methods: To address the mechanistic cross-talk between LRRK2 and GBA1 we generated induced pluripotent stem cells (iPSCs) from fibroblasts obtained from PD patients harboring both the GBA1 N370S and LRRK2 G2019S variants. Isogenic control iPSCs were produced using CRISPR/Cas9 technology to correct the LRRK2 variant, GBA1 variant, or both. These iPSCs were differentiated into microglia (iMGs) and the impact of the LRRK2 and GBA1 variants on lysosomal activity was assessed by measurement of lysosomal protease expression and activity, immunocytochemical analysis of lysosomal morphology, and analysis of phagocytic uptake of exogenous myelin and alpha-synuclein. In addition, the effects of myelin and alpha-synuclein on inflammatory profiles was assessed via measurement of secreted cytokines.

Results: iMGs generated from PD patients harboring both the GBA1 N370S and the LRRK2 G2019S variants displayed aberrant lysosomal morphology with enlarged lysosomes, as well as reduced lysosomal protease activity as measured by DQ-BSA. However, preliminary analysis showed that iMGs harboring both the GBA1 N370S and LRRK2 G2019S variants displayed higher lysosomal GCase enzymatic activity compared to isogenic single GBA1 mutant iMGs. Evaluation of a panel of secreted cytokine demonstrated no genotype-specific differences in cytokine levels in iMGs treated with alpha-synuclein or myelin.

Conclusions: Examination of iPSC-derived microglia harboring dual pathogenic mutations in the GBA1 and LRRK2 genes revealed that double mutant iMGs display multiple defects in lysosomal function. However, lysosomal GCase activity appears to be higher in double mutant iMGs compared to GBA1 single mutant iMGs, suggesting that LRRK2 G2019S may mediate a possible compensatory increase in lysosomal GCase activity in microglia.

Robust and multi-mechanistic Enhancement of Neuronal Autophagy to Rescue Parkinson Disease

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BACKGROUND: Parkinson disease (PD) is an incurable and neurodegenerative movement disorder imparting disabilities on patients due to progressive motor and non-motor symptoms. Degeneration of susceptible neurons in PD is related to toxic misfolding and aggregation of the protein alpha-synuclein (asyn), exacerbated by a decline in the protein-clearance pathway of autophagy. Although pharmacologic stimulation of autophagy is a putatively attractive therapeutic intervention, this strategy fails to produce rescue effects due to neuronal resistance to these stimulatory agents, due to genetic and regulatory inhibitory effectors that are enriched in PD neurons. In particular, we found that an autophagy axis operating through the transactivator, TFEB, and the chaperone, BAG3, were hypofunctional in human neurons with PD pathology. However, the optimal strategy for leveraging this pathway to rescue neurodegeneration remains undefined.

METHODS: To study the molecular pathobiology of PD and candidate therapy strategies with unprecedented detail, we stably integrated a new Tet-ON rapid differentiation cassette into the genomes of human induced pluripotent stem cells (iPSCs) to enable efficient (>99%) differentiation into mature dopaminergic neurons (iDNeurons) within 14 days. To model PD, we used iPSCs expressing a homozygous mutation in SNCA (c.209G>A, p.A53T), or a heterozygous GBA1 mutation (c.1226A>G, p.N370S). To potentiate the autophagy, we used lentiviral delivery of CRISPRa to modify genetic targets of interest, then assessed effects on

asyn, autophagy substrates, mitochondrial health, and cell viability.

RESULTS: In WT and PD iNeurons, overexpression of TFEB or BAG3 lead to significant clearance of both autophagy substrates LC3-II and p62, but also oligomeric and aggregated forms of asyn in PD neurons, and improved markers of mitochondrial homeostasis. Overexpression of these factors also increased cell survival in PD iNeurons. Genetic knockdown of these factors exacerbated PD pathology in WT neurons. A screen of pharmacologic inducers of neuronal autophagy that enhancing expression of these factors also produced similar phenotypic rescue in PD neurons. These effects were abrogated by bafilomycin, but not bortezomib.

CONCLUSIONS: Genetic and pharmacologic enhancement of the TFEB-BAG3 pathway not only promoted autophagic degradation of toxic forms asyn, but also rescued neurodegeneration in multiple heritable forms of PD. Together, our findings inform discrete and specific molecular targets of high translational potential for therapy development for PD and related synucleinopathies.

Neurophysiologic Signatures of Essential Tremor and Implications for Deep Brain Stimulation

Presenting Author: *Lauren Hammer, MD, PhD, University of Pennsylvania*

Background: Essential tremor (ET) is among the most common movement disorders, yet the network-level mechanisms underlying tremor generation and therapeutic response to deep brain stimulation (DBS) remain incompletely understood. Emerging sensing-enabled DBS technologies provide new opportunities to investigate oscillatory activity within the cerebello-thalamo-cortical network and its modulation by DBS.

Objective: To develop and validate multimodal neurophysiologic approaches for identifying neural signatures of ET and characterizing the effects of DBS on tremor-related brain networks.

Methods: As part of an ongoing NIH K23 project, we are integrating thalamic local field potential recordings from sensing-enabled DBS systems with scalp electroencephalography (both cortical and cerebellar), wearable tremor measurements, and quantitative clinical assessments in patients with ET. Methodologic efforts have focused on developing and validating synchronization approaches across independently acquired neural, behavioral, and wearable data streams to enable temporally precise analyses of brain-behavior relationships. In parallel, intraoperative ultra-high-density micro-electrocorticography (microECoG) recordings are being used to characterize cortical network dynamics during DBS implantation procedures.

Preliminary Results: We established a multimodal acquisition and analysis platform capable of synchronizing chronic DBS sensing, neurophysiologic recordings, wearable sensor data, and clinical measurements. Validation studies demonstrated reliable temporal alignment across data streams, providing the foundation for investigating relationships between neural activity and tremor behavior. Initial intraoperative recordings further demonstrate the feasibility of high-resolution cortical neurophysiology during DBS surgery and reveal spatially heterogeneous oscillatory activity across primary motor cortex at sub-centimeter spatial scales.

Conclusions: Development of synchronized multimodal neurophysiology platforms enables detailed investigation of cerebello-thalamo-cortical network dynamics in ET. Ongoing studies will leverage these methods to identify neurophysiologic biomarkers of tremor and improve understanding of DBS mechanisms, with the long-term goal of informing physiology-guided and adaptive neuromodulation strategies.

Somatosensory Gating Impairment in Adults with Chronic Tic Disorder

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BACKGROUND: Nearly two-thirds of children and adults with chronic tic disorder (CTD) experience tactile over-responsivity, defined as heightened awareness of and/or behavioral reactivity to commonplace tactile stimuli. In CTD populations, tactile over-responsivity is associated with more severe tics and psychiatric symptoms, as well as worse quality of life. The neurobiological mechanisms underpinning tactile over-responsivity in CTD are unclear, but somatosensory gating impairment is implicated. Somatosensory gating is the physiological process of filtering out repetitive tactile stimuli at early perceptual stages. In the current study, we sought to determine if adults with CTD exhibit impaired somatosensory gating and if somatosensory gating indices are associated with tactile over-responsivity in this population.

METHODS: Neurotypical controls and adults with CTD completed rating scales assessing tactile responsivity (tactile items of Sensory Perception Quotient, SPQ-Tactile), tics, and psychiatric symptoms. Participants were then monitored with dense-array EEG during a somatosensory gating paradigm, during which pairs of non-noxious, pneumatic stimuli (25 kPa, 20 ms-duration, 500 ms inter-stimulus interval) were repeatedly administered to the fingerpad of the non-dominant hand second digit (100 trials, 7-7.5 s inter-trial interval). Analyses focused on EEG data from a 10-electrode cluster overlying sensorimotor cortex contralateral to the pneumatic stimulus. Using event-related spectral perturbation analysis, frequency band (i.e., delta 1-4 Hz, theta 4-8 Hz, alpha 8-12 Hz, beta 12-30 Hz, and gamma 30-55 Hz) power was calculated for 100-ms epochs following first and second pneumatic stimuli (S1 and S2, respectively). To contrast band power between groups, we used multiple linear regression analyses, comparing nested models with and without CTD status; models included age, sex, and psychiatric comorbidity status as covariates.

RESULTS: To date, data have been collected for 37 neurotypical (14 M: 23 F, 33.8±12.9 years) and 33 CTD (14 M: 19 F, 31.9±9.5 years) adults. Both groups show reduced deviation from baseline in delta, theta, beta, and gamma power after S2, relative to S1, consistent with gating within each of these frequency bands. Multiple between-group differences are evident in beta and gamma bands. After adjusting for covariates, adults with CTD exhibit: smaller decrease in beta power (i.e., reduced beta desynchronization) in the 100 ms prior to S2 (Wald $\chi^2(5)=11.9$, $p<0.05$, $R^2=0.13$; $\Delta R^2=0.07$), consistent with aberrant processing in anticipation of a predictable stimulus; increased gamma power in the 400 ms after S2 (range of results from gamma band models across these epochs: Wald $\chi^2(7)=16.7-26.0$, all $p<0.05$, $R^2=0.31-0.44$; $\Delta R^2=0.09-0.20$), consistent with impaired gamma band gating; and larger decrease in beta power (i.e., increased beta desynchronization) 300-500 ms after S2 (Wald $\chi^2(7)=23.5-39.9$, all $p<0.01$, $R^2=0.29-0.43$; $\Delta R^2=0.07-0.11$), consistent with impaired beta band gating. Additional between-group differences are evident in other bands, after S1 (theta 0-200 ms) and S2 (delta 400-500 ms). Among CTD adults, increased alpha power in the 100-200 ms epoch post-S1, the time window most strongly linked with tactile awareness, is associated with greater self-reported tactile responsivity ($|rs|=0.37$, $p<0.05$).

CONCLUSION: Preliminary findings reveal impaired gating of beta and gamma band activity in response to non-noxious tactile stimuli in adults with CTD. Enrollment is ongoing to ensure the study sample size permits sufficiently powered analyses to address the study's hypotheses.

Exercise for Cognitive Excellence in Parkinson's Disease (EXCEL-PD): A randomized-controlled trial of a home-based, multimodal exercise intervention in people with Parkinson's disease

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Background: Cognitive impairment begins early in Parkinson's disease (PD) and progresses to dementia in most people with PD, reducing quality of life and contributing to growing health-related costs. Physical exercise has potent anti-aging effects and improves many outcomes in PD including cognition. Identifying biomarkers that respond to exercise, and how they associate with cognition and underlying disease pathology, may elucidate key mechanisms for countering cognitive decline.

Objectives: This is a pilot clinical trial that will test the feasibility, adherence, and safety of a 26-week combined endurance and resistance exercise intervention in people with PD. It is also a randomized-clinical trial with secondary objectives to test the efficacy of the exercise intervention on (1) global cognition (2) motor symptom progression and (3) circulating fluid-based biomarker levels.

Methods: Thirty-one participants with PD were randomized to either a home-based, trainer-supervised, combined endurance and resistance training program (exercise group) or a wait list control group for 26 weeks. Feasibility will be assessed using average percentage of maximum heart rate for aerobic exercise and repetition maximums for resistance exercise. Adherence will be assessed using average duration of exercise at target heart rate intensity for aerobic exercise and average days per week and duration of exercise for resistance exercise. Safety will be assessed using adverse events and serious adverse events. Additionally, preliminary efficacy of the combined endurance and resistance exercise intervention will be evaluated by measuring change at 26 weeks from baseline in global cognition and domain-specific cognitive functions, the Movement Disorders Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) motor subscore, and various participant-reported outcomes. Biomarkers of aging (klotho) and stress (cortisol) in periphery (blood, saliva) and brain (cerebrospinal fluid) will also be measured at baseline at 26 weeks.

Results: Recruitment commenced in July 2023 and concluded in November 2025. The last participant completed the study in May 2026. A total of 80 participants were screened for the study, 49 participants were excluded either due to not meeting eligibility criteria or declined participation. Of the 31 total participants enrolled in the study, 16 were randomized to exercise group and 15 to control group.

Conclusions: Previous studies have shown that high intensity endurance exercise effectively slows the progression of motor symptoms in PD, and that resistance exercise effectively improves cognition in PD. Establishing whether a clinically relevant, combined endurance and resistance exercise intervention is safe and feasible in PD, and can improve cognition and slow motor disease progression would have a significant impact on quality of life for those with PD and their caregivers. Understanding how biomarkers respond to exercise will shed important mechanistic insight.

Dissecting Neural Circuits Regulating Sleep State Transitions in a Mouse Model of Parkinson's Disease

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Background: Parkinson's disease (PD) is a common neurodegenerative disease and significant source of disability. While PD is classically defined by motor symptoms, up to 90% of PD patients experience sleep disturbances, which profoundly impact quality of life. Sleep fragmentation – increased switching between sleep and wake states – is a core feature of sleep dysfunction in PD and progressively worsens over the course of disease. The basal ganglia are an interconnected set of subcortical nuclei known to be central to the motor phenotype seen in Parkinson's disease, but circuits underlying sleep fragmentation in PD are less clear. It has therefore been challenging to target treatments for sleep symptoms in PD. The goal of this study is to examine circuit mechanisms of sleep fragmentation in a dopamine depletion model of PD.

Methods: Mice underwent unilateral stereotaxic injection of the neurotoxin 6-hydroxydopamine (6-OHDA) or saline in the medial forebrain bundle to generate parkinsonian mice and controls. Parkinsonian mice and controls were then implanted with EEG and EMG

recording devices for continuous recording of sleep. Sleep states were classified as either Wake, REM, or NREM based upon spectral analysis. The number of sleep state transitions per minute were calculated as a measure of sleep fragmentation.

Results: Mice injected with 6-OHDA show near complete loss of dopamine neurons. Parkinsonian mice demonstrated decreased movement velocity and rotate ipsilateral to the injected side compared to saline treated controls. We found that parkinsonian mice exhibited more frequent sleep-wake transitions, indicative of increased sleep fragmentation, compared to saline treated controls.

Conclusions: Many of the sleep changes observed in people with PD are attributed to progressive neuropathology in brainstem regions regulating sleep. In this study, dopamine depleted mice demonstrated robust sleep fragmentation. This result suggests that sleep fragmentation may be related to circuit dysfunction downstream of dopamine loss, rather than directly due to underlying pathology in other brain areas. Future work will examine how changes in basal ganglia activity may contribute to sleep fragmentation.

Dissecting The Mechanisms Of BORCS5-Related Lysosomal Dysfunction

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Background: BORCS5 encodes a subunit of the BORC complex, a lysosome-associated multi-protein assembly that mediates ARL8/kinesin-dependent anterograde lysosome transport. We previously demonstrated that pathogenic BORCS5 variants cause a spectrum of neurodevelopmental and neurodegenerative disorders. While truncating variants abolish BORCS5 protein expression and impair anterograde lysosome movement, the missense variants R95Q and H99P preserve peripheral lysosome distribution in patient fibroblasts and iPSC-derived neurons. Nevertheless, both complete BORCS5 loss and missense variants disrupt lysosome activity. However, the mechanisms by which BORCS5 and its pathogenic missense variants contribute to lysosomal dysfunction and neuronal pathology remain unknown. Here, we aim to characterize the pathological mechanisms underlying BORCS5 missense variants and elucidate the role of BORCS5 in lysosome homeostasis beyond its canonical function in anterograde lysosomal transport.

Methods: To dissociate the role of BORCS5 in lysosome homeostasis from its role in anterograde transport, we generated the following cellular models: (1) HeLa knock-in (KI) cell lines homozygous for R95Q or H99P; (2) BORCS5 knockout (KO) HeLa cells stably re-expressing wild-type (WT) BORCS5; and (3) BORCS5 KO HeLa cells stably expressing KBS-LAMP1, a synthetic construct comprising three SKIP-binding kinesin motifs (KBS) fused to LAMP1, which restores anterograde lysosome movement independently of BORC. Additionally, BORCS5 KO and R95Q/H99P KI iPSCs stably expressing NGN2 were differentiated into induced neurons (iNeurons). Lysosomal proteolytic activity was assessed by DQ-BSA assay via flow cytometry (HeLa cells) or live-cell imaging (iNeurons). Lysosome distribution, axonal lysosome trafficking, and morphology in mature iNeurons were examined by confocal microscopy, live-cell microscopy, and transmission electron microscopy (TEM). Lysosomal proteomes will be profiled using lysosomal immunoprecipitation (LysolIP) coupled with TMT-based quantitative proteomics of both lysosomal and whole-cell fractions.

Results: As anticipated, BORCS5 KO caused perinuclear collapse of the lysosome network and elevated LC3-II levels in HeLa cells, indicating impaired lysosomal trafficking and autophagosome clearance. In contrast, R95Q and H99P KI cells maintained normal lysosome distribution and LC3-II levels. However, all three genotypes — KO, R95Q, and H99P — exhibited reduced lysosomal proteolytic efficiency by DQ-BSA assay, demonstrating that the missense variants impair lysosome function despite preserving anterograde movement. To further dissociate transport from function, we expressed BORCS5-WT or KBS-LAMP1 in KO cells. Both constructs restored anterograde movement; however, only BORCS5-WT rescued LC3-II levels and recovered cathepsin B (CTSB) and glucocerebrosidase (GCase) protein levels, indicating that restoring lysosome positioning alone is insufficient to correct lysosomal dysfunction and autophagy impairment caused by BORCS5 loss. In iNeurons, BORCS5 KO produced predominantly immobile, enlarged lysosomes confined to the soma, while R95Q/H99P KI iNeurons showed near-complete recovery of axonal lysosome trafficking. BORCS5 KO iNeurons exhibited several features of lysosomal dysfunction, including reduced GCase activity, decreased CTSB and GCase protein levels, elevated LC3-II and LAMP1/2, and autophagosome accumulation by TEM — consistent with impaired autophagosome clearance. LysolIP confirmed depletion of CTSB and GCase within endolysosomal fractions of KO iNeurons.

Conclusions: BORCS5 depletion and bi-allelic variants lead to lysosomal dysfunction. BORCS5-depleted cells exhibit reduced lysosomal proteolysis and decreased CTSB and GCase levels and activity, which cannot be rescued by restoring anterograde lysosome movement, indicating that BORCS5 plays a role in lysosome homeostasis beyond its canonical function in anterograde transport. Quantitative proteomics of whole-cell and lysosomal fractions is ongoing to comprehensively define how BORCS5 loss and missense variants remodel the lysosomal proteome.

Projections from Hippocampal CA1 to Striatum are Biased Toward Either the Striosome or Matrix Compartments

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Background: Hippocampo-striate projections are essential for linking spatial and contextual memory with action selection, reward prediction, and goal-directed behavior. The hippocampus routes information to the striatum through polysynaptic circuits,

innervating medium spiny neurons in one of two distinct tissue compartments, the striosome or matrix. Though the compartments are interdigitated throughout the striatum, they differ in their embryologic origins, relative abundance, intra-striate location, and afferent and efferent connectivity. We recently demonstrated in humans that striosome and matrix have markedly different functional connectivity networks and are engaged in different phases of tasks. Therefore, hippocampo-striate projections that synapse in striosome may underpin different functions and engage with different functional networks than inputs that synapse in matrix. However, it was unknown whether hippocampo-striate projections are organized differently for striosome and matrix, or if compartment-specific organization differed between polysynaptic paths.

Methods: We used connectivity-based parcellation in healthy adults (probabilistic diffusion tractography, N=930, Human Connectome Project) and anterograde viral injections with subsequent immunohistochemistry in mice to assess structural and functional connectivity between CA1 and the striatal compartments. We identified subject-specific CA1, striosome-like, and matrix-like masks in each human subject based on structural and diffusion MRI-based parcellations. We defined three hippocampo-striate pathways, each constrained to pass through one of three intermediary nuclei (subiculum, medial prefrontal cortex, or lateral septum) with stringent selection for anatomically-validated streamlines. In mice, we injected the viral tracer YFV-17D in dorsal or ventral CA1. After eight days (optimized to identify hippocampo-striate projections) we quantified immunofluorescent soma within striosome or matrix using calbindin immunohistochemistry.

Results: In mice, each CA1 viral injection labeled projections that were either striosome-favoring or matrix-favoring – none were unbiased. Therefore, our viral vector's diffusion diameter (~1mm) appeared to be enclosed within segregated somatotopic zones that preferentially projected to only one striatal compartment. Consistent with this finding, in humans we identified discrete somatotopic zones, interspersed throughout CA1, that were biased either toward striosome- or matrix-like voxels. Striosome-biased CA1 volume was 3.5-fold larger than matrix-biased volume. Each hippocampo-striate path was striosome-biased (medial prefrontal > subiculum > lateral septum), but the somatotopic zones for each hippocampo-striate path were largely segregated from the zones of other paths (overlap, two-paths: 5.7%; overlap, three-paths: 0.12%). Projections to randomly-selected striatal voxels (negative control) lost this bias. Resting state functional activation in CA1 was correlated with striosome-like voxels but anti-correlated with matrix-like voxels.

Conclusions: Hippocampo-striate projections may be segregated into distinct networks that serve striosome- or matrix-specific functions.

Neurocritical Care and Traumatic Brain Injury

Time-Dependent Remodeling of the Choroid Plexus Promotes Neutrophil Recruitment After Traumatic Brain Injury

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Background: Acute inflammation is a major contributor to secondary neurological injury after traumatic brain injury (TBI). The barriers that separate the brain from the systemic circulation are major sites for inflammatory activation and transit points for immune cells into the central nervous system. The choroid plexus is one such barrier and has been shown to play a major role in inflammation in a variety of brain disorders. The choroid plexus may be amenable to therapeutic modulation through treatments directed into the cerebral ventricles. In TBI, such therapeutic advances are hampered by our poor understanding of the role of the choroid plexus. We sought to elucidate time-dependent changes in the choroid plexus in controlled cortical impact, a common model of TBI.

Methods: Male C57BL/6 mice (age 7-12 weeks) underwent controlled cortical impact, a focal hemorrhagic contusion model of traumatic brain injury. Sham craniectomy was used as a control. Downstream analyses were performed on lateral ventricle choroid plexuses harvested at various time points after injury. Bulk RNA sequencing was performed on RNA extracted at 3 hours, 6 hours, and 24 hours after injury. As an additional control in bulk RNA sequencing analysis, we compared transcriptomes to those obtained from choroid plexus in a model of intraventricular hemorrhage. Luminex analysis for proteins CXCL1 and CXCL2 was performed on protein lysates obtained at 6 hours after injury. Immunohistochemistry to label the neutrophil marker myeloperoxidase (MPO) was performed on sections prepared from tissue harvested at 24 hours after injury. Single-cell RNA sequencing was performed on single-cell suspensions obtained at 24 hours after injury.

Results: We found a time-dependent, lateralized remodeling of the choroid plexus transcriptome after TBI. This transcriptome was distinct from that observed after intraventricular hemorrhage. The TBI-induced choroid plexus transcriptome was characterized by early transcriptional upregulation of inflammation-associated transcription factors and several key cytokines and chemokines. Most notably, the two most upregulated genes in the transcriptome by 6 hours after injury were *Cxcl1* and *Cxcl2*, which encode CXC-motif chemokines that are powerful neutrophil chemoattractants. We validated the upregulation of CXCL1 and CXCL2 at the protein level using Luminex. By 24 hours, there was upregulation of a several genes that encode proteins involved in extracellular matrix remodeling. Upregulated transcripts were enriched in targets of several transcription factors most notably NF- κ B. Consistent with these changes, we observed a

significant influx of neutrophils into the ipsilateral lateral ventricle choroid plexus at 24 hours after injury, using immunohistochemistry. To further delineate cell-type specific changes in the choroid plexus transcriptome, we performed single-cell RNA sequencing on cell suspensions prepared from lateral ventricle choroid plexus. We found that greatest TBI-induced changes to the transcriptome were restricted to the choroid plexus epithelial cells. These cells formed two major clusters (distinguished by expression of the long non-coding RNA MALAT1), which exhibited overlapping but distinct transcriptional changes associated with acute inflammation.

Conclusions: In the hours after TBI, the choroid plexus exhibits profound inflammatory changes, most prominently associated with upregulation of CXCL1 and CXCL2 at both the mRNA and protein level. This is accompanied by influx of neutrophils into the choroid plexus. The additional upregulation of extracellular matrix remodeling genes may suggest a modulation of the barrier into the CNS to facilitate the transit of these neutrophils. The transcriptional changes observed appear to occur predominantly in the choroid plexus epithelium. The response of the choroid plexus epithelium is heterogeneous, clustering based on expression of the long non-coding RNA MALAT1. MALAT1 and NF- κ B may serve as regulators of this process, helping to orchestrate a coordinated, time-dependent inflammatory cascade in the choroid plexus after TBI. In the future, we intend to modulate these upstream pathways and block chemokine expression downstream in the choroid plexus. We anticipate that this will reveal novel mechanisms for immunomodulation in the central nervous system that may have therapeutic applications for TBI and other acute neurological disorders.

Distinct Cytokine Trends are Associated with Outcome after Aneurysmal Subarachnoid Hemorrhage

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Background: An inflammatory cascade is activated within the central nervous system following aneurysmal subarachnoid hemorrhage (aSAH). In animal models, the presence of pro-inflammatory cytokines within the cerebrospinal fluid (CSF) is associated with CSF hypersecretion and shunt-dependent post-hemorrhagic hydrocephalus. Additionally, in both animal models of aSAH and in patients, higher concentrations of several pro-inflammatory cytokines are associated with delayed cerebral ischemia (DCI), a devastating form of delayed stroke that occurs in many of these patients, as well as poor neurological outcome. We investigated the relationship between the trajectory of several cytokines within the CSF and outcomes and complications including DCI, shunt-dependent hydrocephalus (SDHC), and poor neurological outcome as measured by the modified Rankin Scale (mRS) at 3 months.

Methods: CSF samples were collected between post-bleed day 0 and 16 from aSAH patients who had external ventricular drains placed as part of their clinical care. We used cytometric bead array assays to analyze the concentration of a panel of cytokines including C-C motif chemokine ligand 2 (CCL2), interleukin (IL)-6, IL-8, IL-10, IL-1 β , IL-4, granulocyte-colony stimulating factor (G-CSF), vascular endothelial growth factor-A (VEGF-A), C-X-C motif chemokine ligand 10 (CXCL10), and tumor necrosis factor- α (TNF- α) in these biological samples. Clinical, demographic, and follow-up data were prospectively collected and adjudicated for accuracy. Poor neurological outcome at 3 months was defined as mRS $\geq$ 3. We investigated the relationship between cytokine trajectories and DCI, SDHC, and 3-month mRS using two complementary approaches. First, we estimated patient-specific cytokine trajectory summaries, including longitudinal intercepts and slopes, and used these features as predictors in logistic regression models, hereafter referred to as momentum models. Second, we used linear mixed-effects models to evaluate how cytokine levels changed over time in relation to each outcome. To assess the most informative post-bleed period, we performed a dynamic time-window analysis in which cytokine trajectory summaries were repeatedly estimated using measurements collected up to different post-bleed days and evaluated using within-sample prediction accuracy and area under the receiver operating curves (AUROC).

Results: 91 patients with aSAH (60 $\pm$ 13.7 years old, 68% female) who had >1 CSF sample collected during their hospitalization were included in the analysis. Of these patients, 78 (86%) had available 3-month follow-up data, 77 (85%) had available DCI data, and 12 (13%) had SDHC. In both the momentum and the linear mixed-effects models, higher CSF concentrations of CCL2, IL-6, and VEGF-A over the 2-weeks post-bleed during which CSF was collected is associated with increased likelihood of DCI. Prediction accuracy and AUROC for DCI prediction are highest around post-bleed day 7 for CCL2, day 11 for IL-6, and day 13 for VEGF-A. Higher CSF concentrations of IL-6 and IL-10 are associated with increased likelihood of shunt dependence in the momentum model, and IL-6, IL-10, CXCL10, IL-8, and G-CSF are associated with SDHC in the linear mixed-effects model. For SDHC, prediction accuracy and AUROC are highest in the first week post-bleed (days 5-7) for all significant cytokines. In both the momentum and linear mixed-effects models, higher CSF concentrations of CCL2, IL-6, IL-8, and VEGF-A overall are associated with worse neurological outcome at 3 months, but only CCL2 remains significant after adjustment for DCI. In the dynamic time-window analysis, CCL2 is most predictive of outcome when data is considered from the entire 16-day time window, but when adjusted for DCI the predictive accuracy and AUROC are highest when data through post-bleed day 7 is included.

Conclusions: CCL2 and IL-6 in the CSF of aSAH patients emerged as promising markers of post-aSAH complications and outcomes.

Elevated CCL2 during the first post-bleed week was associated with DCI, while persistently higher CCL2 concentrations over the 2-week post-bleed period were associated with worse neurological outcome. Higher CSF IL-6 was also associated with DCI, with the strongest predictive performance around post-bleed day 11, and IL-6 elevations during the first post-bleed week were associated with SDHC. However, IL-6 was not associated with worse neurological outcome at 3 months. Understanding the temporal dynamics of CSF cytokines after aSAH will enable us to develop targeted immunological treatments and to understand the appropriate timing of treatment to improve outcomes in this devastating form of stroke.

REducing Sympathetic Hyperactivity After Traumatic Brain Injury Using Transcutaneous Auricular Vagus Nerve Stimulation (REST-VNS): A Literature Review, Rationale, And Clinical Trial Protocol

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Background: Sympathetic-parasympathetic imbalance has been associated with worse outcomes after severe traumatic brain injury (sTBI), motivating clinical and translational research targeting dysautonomia to improve patient outcomes. Transcutaneous auricular vagus nerve stimulation (taVNS) offers a non-invasive neuromodulatory alternative to standard pharmacologic approaches. Implementation of taVNS in sTBI to maximize benefit and minimize risk requires careful selection of patients, stimulation parameters, and endpoints. Here, I provide a review and rationale for targeting adult sTBI patients for prophylactic repetitive taVNS to reduce the clinical burden of paroxysmal sympathetic hyperactivity (PSH), a common and severe manifestation of dysautonomia after sTBI, in an early phase 2 clinical trial.

Methods: The REducing Sympathetic hyperactivity after Traumatic brain injury using transcutaneous auricular Vagus Nerve Stimulation (REST-VNS) trial will be a single-center, double-blind, randomized, sham-controlled trial with target enrollment of 50 adults (age 18-65) with sTBI who will undergo one-hour device sessions twice daily beginning 2-5 days post-injury for 5 days (10 sessions) total. The primary outcome will be a between-group difference in prospectively measured PSH-AM score burden on study intervention days 3-5. Secondary outcomes will include immediate physiologic changes in heart rate variability measurements of autonomic balance, PSH-directed treatment intensity level (PSH-TIL) on days 3-5, and safety and feasibility metrics. Exploratory measurements will include biomarkers of inflammation, neural injury, and blood-brain-barrier breakdown, radiographic measures of cerebral edema and blood-brain-barrier breakdown, and clinical outcomes such as duration of mechanical ventilation, length of stay, discharge disposition, Glasgow Coma Scale (GCS), JFK coma scale, and Rancho Los Amigos Scale on discharge.

Expected results: Based on retrospective review of the R Adams Cowley Shock Trauma Center institutional trauma registry, I expect approximately 115 eligible patients per year; target enrollment of 50 patients over 2 years will be feasible with 20-25% enrollment. Based on prior internal data, this will result in >80% power to detect a 4 point difference in the PSH-AM total, a 2 point difference in the PSH-AM CFS subscore, a 1 point difference in PSH-TIL, and an immediate reduction in the LF/HF HRV ratio.

Conclusions: The REST-VNS trial will investigate whether taVNS can reduce the clinical burden of PSH after sTBI via autonomic neuromodulation. If successful, results from this study will guide the design of larger phase 3 trials investigating the intervention's impact on sTBI recovery and healthcare resource utilization.

Impaired Cerebral Autoregulation is Associated with Carbon Dioxide Levels in Pediatric ECMO Patients

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Background: Neurologic injuries are common in pediatric patients who require Extracorporeal Membrane Oxygenation. These injuries incur major morbidity and increase the risk of mortality for these patients. Cerebrovascular autoregulation (CAR) maintains stable cerebral blood flow despite fluctuations in systemic blood pressure. Impaired CAR has been implicated as a risk factor for neurologic injury in pediatric ECMO. The cerebral oximetry index (COx), derived noninvasively from near-infrared spectroscopy and arterial blood pressure correlation, has been proposed as a continuous bedside surrogate for impaired CAR. Blood carbon dioxide (pCO₂) is a potent regulator of cerebrovascular tone known to influence autoregulatory function. In pediatric ECMO, large changes in pCO₂ often occur between pre-ECMO and intra-ECMO periods and have been associated with increased neurologic complications. These associations suggest that abnormal CO₂ mediated impaired CAR contributes to neurologic injury risk. However, the relationship between pCO₂ and CAR during ECMO remains poorly characterized. Establishing whether COx varies systematically with pCO₂ is important both for validating COx as a physiologically meaningful marker of CAR and for identifying potentially modifiable CO₂ targets during ECMO.

Methods: We performed a retrospective observational study of pediatric ECMO patients at a single center. The cohort included all patients aged 0 days through 18 years cannulated onto venovenous (VV) or venoarterial (VA) ECMO with synchronized physiologic monitoring and arterial blood gas data available for analysis. COx metrics were calculated from the 30-minute periods before and after each arterial pCO₂ measurement and included mean COx, percent time with COx > 0.3, and area under the curve (AUC) for COx > 0.3. Repeated observations within patients were analyzed using patient-adjusted regression models with clustered standard errors. To characterize the shape of the association between pCO₂ and COx without imposing arbitrary

thresholds, pCO₂ was modeled continuously using cubic spline regression with knots placed at the 10th, 50th, and 90th percentiles of the observed pCO₂ distribution. Linear and spline model specifications were compared to assess for non-linearity.

Results: The study included 11,106 paired pCO₂-COx observations from 232 pediatric ECMO patients. Median pCO₂ was 43 mmHg (10th percentile 35 mmHg; 90th percentile 56 mmHg). Across all analyses, higher pCO₂ was associated with greater COx burden. Spline modeling demonstrated significant non-linear associations between pCO₂ and mean COx ($p = 0.00035$), percent time with COx > 0.3 ($p = 7.8 \times 10^{-8}$), and AUC for COx > 0.3 ($p = 1.7 \times 10^{-6}$). The cerebral oximetry metric increased on either side of normocapnia (~40mmHg pCO₂). Relative to a reference pCO₂ of 40 mmHg, pCO₂ values of 55 mmHg were associated with increases of +0.040 in mean COx, +5.43 percentage points in time with COx > 0.3, and +1.276 in AUC for COx > 0.3. At roughly a pCO₂ of 60-70, impaired autoregulation burden appeared to plateau.

Conclusions: In pediatric VV and VA ECMO patients, higher pCO₂ was associated with progressively impaired CAR as estimated by COx. The analysis indicates a non-linear relationship characterized by worsening autoregulatory burden above approximately 40-45 mmHg. These findings support the physiologic validity of COx as a dynamic marker of CAR during ECMO and suggest that hypercapnia may represent a modifiable contributor to autoregulatory dysfunction. Continuous COx monitoring may provide a practical bedside approach for individualized assessment of cerebrovascular response to CO₂ management during ECMO. To improve generalizability, a similar analysis utilizing multicenter data is planned.

Hemoglobin Metabolites and Mitochondrial Dysfunction in Traumatic Brain Injury

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Background: Following traumatic brain injury (TBI), a significant number of patients have associated intracranial hemorrhage. After injury, red blood cells lyse releasing hemoglobin (Hgb) and its breakdown product hemin. These byproducts have been shown to cause significant oxidative damage. TBI associated hemorrhage may lead to mitochondrial oxidative stress, cell death, and behavioral impairment.

Methods: Human induced pluripotent stem cell (iPSC)-derived neurons were incubated with hemin or iron. Lipid peroxidation was assessed using BODIPY 581/591 C11 dye, and mitochondrial depolarization was assessed using JC-1 dye. Mitochondrial iron levels were assessed using Mitoferro Green. Additionally, using a mouse model of TBI, free iron producing enzyme (heme oxygenase) and lipid peroxidation markers of immunohistochemistry were assessed in the cortex adjacent to contusion injury were assessed.

Results: In iPSC-derived neurons, increased lipid peroxidation, mitochondrial depolarization, and mitochondrial iron was detected after hemin or iron administration. In the mouse model of TBI, increases in heme oxygenase and lipid peroxidation was detected at the contusion site.

Conclusions: TBI associated hemorrhage may lead to accumulation of mitochondrial iron, which exacerbates TBI-induced mitochondrial oxidative stress and injury. In the future studies, we will use adeno-associated virus (AAV) vector delivery of mitochondrial ferritin (FtMt) gene to the cortex to mitigate oxidative injury and mitochondrial dysfunction in TBI. Additionally, we will assess if reducing mitochondrial lipid peroxides or hydrogen peroxide in TBI will attenuate histological measures of injury and behavioral dysfunction following TBI. This will be accomplished using AAV to express mitochondrial catalase (mitoCAT) or Peroxiredoxin-3 (Prdx3).

Cardiac Arrest Stakeholders' Views on Measuring Prognostication Quality in Post-cardiac Arrest Patients: A Concept Mapping Study

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Background: It is unclear how to evaluate the quality of clinicians' outcome predictions for patients treated after cardiac arrest. Past research has focused on sensitivity and specificity for predicting poor outcomes, but other measures may be preferable. Because a perceived poor prognosis often leads to withdrawal of life-sustaining treatments, accuracy cannot be used.

Methods: We performed a concept mapping study, a mixed-methods stakeholder engagement strategy, to both identify and rank potential metrics of prognostication quality. We purposively recruited cardiac arrest stakeholders from unique backgrounds and perspectives: healthcare professionals (physicians, nurses, social workers), family members of survivors (who recovered to varying degrees), family of non-survivors, survivors (who had varying hospital experiences), and domain experts in religion/spirituality and ethics. We conducted three pilot focus groups and individual sessions to refine presentation material and prompt for comprehension and clarity across stakeholder groups. Following piloting, we performed 22 stakeholder sessions that included an

introductory presentation and then collected individually enumerated potential measures of prognostication quality after cardiac arrest. We (AS, KJR, ML) removed irrelevant concepts, consolidated duplicative responses, and clarified concepts. We gave this synthesized list of measures back to the same stakeholders to sort concepts based on similarity and rank them by importance. We then generated graphic representations of the results. We will present the graphic results to stakeholders, integrate feedback, and refine results as needed.

Results: We included 69 cardiac arrest stakeholders (18 physicians, 3 nurses, 4 social workers, 14 cardiac arrest survivors, 16 family members of survivors, 7 family members of non-survivors, 9 ethics experts, and 7 spirituality/religion experts). Participants' median age was 48 years (IQR 40-61), 42 (61%) were female, 56 (81%) were white, and 64 (88%) were non-Hispanic. Stakeholders enumerated 443 statements (measures), which we reduced to 125 distinct concepts. Common potential measures could be categorized into reproducibility, transparency, bias, timing, evidence and tests, care team processes and experience, reassessment of prediction, fairness and uncertainty, and ways to review prediction quality (i.e., artificial intelligence, prediction models, guidelines, hospital protocols/checklists, expert panels).

Conclusion: Stakeholders identified numerous potential measures of prognostication quality. Many are novel and rarely used in prognostication literature. Some factors focus on intangible components of prognostication, whereas others, identified as highly important, could be used as key outcome measures to assess clinical practice and test prognostication interventions.

Probing and Promoting Brain Injury Recovery with Non-Invasive Thalamic Focused Ultrasound

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Background: Disorders of consciousness (DoC) following severe brain injury are associated with profound impairments in arousal, awareness, communication, and meaningful interaction. Despite advances in understanding thalamocortical network dysfunction after severe brain injury, few validated therapies exist to promote recovery of consciousness. Low-intensity focused ultrasound pulsation (LIFUP) is a promising non-invasive neuromodulation approach capable of targeting deep brain structures, including the thalamus. This NIH-funded K23 study evaluates thalamic LIFUP as a potential therapeutic and mechanistic probe in patients with DoC, while integrating neuroimaging and neurophysiologic biomarkers with surrogate/family perspectives in an early-phase clinical development framework.

Methods: This is an ongoing prospective study enrolling patients with DoC after severe brain injury to receive non-invasive thalamic LIFUP. Participants undergo serial standardized neurobehavioral assessments to evaluate changes in level of consciousness and recovery trajectory. Resting state and task-based EEG is acquired before and after LIFUP to assess neurophysiologic response, with emphasis on changes in connectivity and reactivity within consciousness-relevant networks. In parallel, semi-structured qualitative interviews are conducted with patient representatives, including family members and surrogate decision-makers, to characterize ethical perspectives on therapeutic neuromodulation research involving patients who cannot provide first-person consent.

Results: Enrollment has begun and remains ongoing. To date, the study has demonstrated the feasibility of initiating an integrated prospective protocol combining neuroimaging-guided thalamic LIFUP, serial standardized neurobehavioral assessment, pre- and post-stimulation EEG, and embedded neuroethics through qualitative interviews with patient representatives. This protocol establishes a translational platform for testing neuroimaging-based predictive biomarkers and EEG-based pharmacodynamic endpoints in early-phase clinical trials for brain injury. In parallel, the study has yielded an initial ethical framework to guide responsible investigation of LIFUP and related neuromodulation technologies in patients with DoC, with further refinement planned as qualitative data accrue.

Conclusions: This ongoing study will provide foundational data on the feasibility, neurobehavioral effects, neuroimaging-based predictive biomarkers, EEG-based pharmacodynamic endpoints, and ethical dimensions of low-intensity focused ultrasound neuromodulation for patients with disorders of consciousness after brain injury. By integrating serial behavioral assessment, connectome-informed targeting, EEG-based measures of target engagement, and surrogate/family perspectives, the project will inform the design of larger trials of personalized neuromodulation for impaired consciousness and cognition after brain injury. This work supports an ethically grounded translational pathway for restorative neurotechnology in a population with substantial unmet therapeutic need.

Neurodegeneration and Cell Death

Regional and Age-Related Differences in Lysosomal pH in the Mouse Brain Revealed by a Genetically Encoded Sensor

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Background: Lysosomal dysfunction is increasingly implicated in age-related neurodegenerative disorders. The lysosome is an acidic, catabolic organelle responsible for degrading waste and misfolded proteins, and its function depends on maintaining a low luminal pH. While studies in yeast and *C. elegans* have shown that lysosomal pH increases with age, it remains unclear whether similar changes occur in mammalian systems.

Methods: To address this, we developed a novel mouse model expressing a genetically encoded lysosomal pH sensor, Fluorescent Indicator REporting pH in Lysosomes (FIRE-pHly). This ratiometric reporter is targeted to lysosomes and consists of a cytosolic, pH-insensitive mCherry domain and a luminal, pH-sensitive mTFP fluorophore ($pK_a = 4.5$), optimized for measuring lysosomal pH. Using an inducible floxed promoter design, FIRE-pHly expression can be selectively driven in the brain with cell type-specific Cre lines.

Results: In young adult FIRE-pHly mice, we observed regional heterogeneity in neuronal lysosomal pH. Notably, brain regions known to be vulnerable in neurodegenerative diseases exhibited relatively elevated lysosomal pH at baseline. To our knowledge, this is the first in vivo demonstration of region-specific differences in lysosomal pH in the mammalian brain, identifying a previously unrecognized physiological variable that may contribute to selective neuronal vulnerability. Furthermore, viral-mediated expression of FIRE-pHly in aged mice revealed increased lysosomal pH in the cortex compared to younger controls, consistent with age-associated lysosomal alkalinization in mammalian tissue.

Conclusions: Together, these findings establish FIRE-pHly as a versatile in vivo tool for quantifying lysosomal pH in the brain and suggest that both regional and age-related differences in lysosomal pH warrant further investigation in the context of neurodegenerative disease.

Diffusion Tensor Imaging as a Data-Driven Classifier of Early Cerebral Adrenoleukodystrophy

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Objective: Childhood cerebral adrenoleukodystrophy (CALD) is a rapidly progressing neurological disorder that must be treated early to prevent neurocognitive decline. In current clinical practice, life-saving cellular therapies are initiated only after white matter changes from CALD appear on structural MRI. Our objective was to evaluate the sensitivity and specificity of region-based metrics from brain diffusion tensor imaging (DTI) for classifying and predicting early-stage CALD.

Methods: We analyzed a diffusion MRI dataset derived from scans of 119 boys with adrenoleukodystrophy (ALD) acquired between 2007–2025. Images were processed from 74 (62%) boys with CALD scanned prior to treatment and 45 (38%) boys with ALD and no lesion (NL-ALD). Of the CALD patients, 5 had sequential scans acquired 3–27 months before lesion development (“pre-lesion ALD”; PL-ALD). Across all subjects, 266 total scans were categorized: CALD ($n=87$), PL-ALD ($n=13$) and NL-ALD ($n=166$). Single-shell DTI ($b=1000$ s/mm²) was acquired using two protocols (12-directional with voxel size $2 \times 2 \times 5$ mm³ and 66-directional with voxel size $2 \times 2 \times 2$ mm³). Fractional anisotropy (FA), radial, axial, and mean diffusivity (RD, AD, MD) were estimated for 57 atlas-defined white matter regions. Stepwise linear regression, stratified by age and DTI protocol, identified a minimal DTI feature set that predicted disease status (C-ALD, PL-ALD, or NL-ALD). To determine whether a logistic regression classifier could differentiate CALD from NL-ALD scans using the identified DTI feature set, cross-validation was performed with a 75%-25% train-test split with no patient overlap between datasets (5,000 permutations). CALD lesion severity was quantified using a disease-specific radiological scoring system (Loes score).

Results: The NL-ALD cohort's baseline age (7 ± 3 years) was slightly lower than that of the CALD cohort (8 ± 2 years), $p=0.08$. The median pre-treatment Loes score among CALD patients was 3.0 (IQR 2.0–7.5; range: 1.0–21.5). Stepwise regression identified 85 DTI features, independent of age and DTI protocol, with a Pearson correlation of $r=0.98$ between predicted and actual disease status. In cross-validation, the logistic regression classifier identified CALD scans with 100% sensitivity (95% CI: 86–100%) and 100% specificity (95% CI: 95–100%). Notably, DTI features were present at the pre-lesion stage before a visible T2-weighted MRI lesion developed.

Conclusion: Using an automated algorithm, multi-region DTI analysis achieved high classification accuracy in differentiating scans with and without CALD lesions. Crucially, DTI features could detect white matter microstructural changes not yet evident on conventional MRI. This suggests that DTI could serve as a primary “early warning” biomarker to optimize treatment timing.

Neurodevelopment

The BAF Complex Works with FOS To Regulate Human Neuronal Activity-Dependent ASD-Associated Gene

Programs

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Abstract: The BAF chromatin remodeling complex is critical to normal brain development, and rare variants within genes encoding BAF subunits are a common genetic cause of neurodevelopmental disorders, including autism spectrum disorder (ASD). Yet the factors that direct BAF binding across the neuronal genome, the human neuronal gene programs that are regulated by BAF, and the mechanisms whereby BAF subunit perturbation leads to ASD are not known. We find that BAF binds with the activity-dependent transcription factor FOS to distal regulatory regions that, in response to neuronal activity, undergo chromatin opening and show evidence of enhancer activation. Knock-out of ARID1A, a BAF subunit implicated in ASD, leads to decreased chromatin accessibility at FOS/BAF binding sites concomitant with decreased expression of nearby activity-regulated ASD-associated genes. Additionally, we find that the FOS binding motif in FOS/BAF-bound regions is highly constrained in the human population, and that rare variants in this motif in ASD-affected individuals disrupt stimulus-dependent enhancer activation. This suggests that genetic variation in FOS/BAF-bound regions contributes to ASD pathogenesis, due to an inability to recruit FOS and BAF to enhancers to promote gene expression. Together, our findings highlight a role for BAF in mediating neuronal transcriptional programs downstream of FOS and reveal a mechanism by which non-coding variants may impact BAF function and contribute to risk for ASD.

Neurogenetics and Gene Therapy

Longitudinal Sway and Gait Changes and Discrimination of Fall Risk in Adults With X-Linked

Adrenoleukodystrophy

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Background: X-linked adrenoleukodystrophy (ALD) is the most common leukodystrophy and peroxisomal disorder, with a prevalence of approximately one in 14,000–17,000 live births. The clinical phenotype is heterogeneous and includes adrenal insufficiency, myeloneuropathy, and cerebral demyelination. Myeloneuropathy is the most prevalent presentation and develops in all affected men and in 80-90% of women, although disease severity is generally greater in men due to X-linkage. There are no FDA-approved treatments for myeloneuropathy. Quantifying disease progression in X-linked adrenoleukodystrophy (ALD) remains a bottleneck for clinical trials due to low sensitivity of conventional functional scales. We tested whether instrumented sway and gait from body-worn inertial sensors (Opal/APDM) change over two years and discriminate fallers from non-fallers in men and women with X-ALD myeloneuropathy.

Methods: Sway was recorded in four standing paradigms (feet apart/together × eyes open/closed). Gait was recorded during the 6-MWT at two academic medical centers. Features were modelled with linear mixed-effects models adjusted for sex and walking-aid use, with Benjamini–Hochberg correction within each variable × paradigm pair. Baseline AUC thresholds defined an anchored minimal clinically important difference (MCID). Patients were classified into four MCID trajectories: Stayed Below-, Stayed Above-, Crossed Into-, or Crossed Out of risk.

Results: 118 patients (68 male, 50 female; mean age 36.5 years men, 52.5 years women) had a fully assessable baseline. After Benjamini–Hochberg-correction, 18 pairwise sway contrasts were significant pooled (4 male, 12 female), concentrated in eyes-open paradigms. Gait performance declined over two years (speed –0.083 m/s; stride length –0.059 m; both $p < 0.001$). Baseline feet-together eyes-closed sway discriminated patients who reported falls at any frequency (any-fallers) with AUCs up to 0.88 (23/76 pairs ≥ 0.80). Patients above the Sway Area MCID at both baseline and 2-year follow-up visits had near-100% any-faller rates versus near-0% below (BH-corrected $p = 0.012$).

Conclusion: Eyes-open sway paradigms detect longitudinal change over 2-years in both men and women with X-ALD myeloneuropathy. Eyes-closed sway paradigms discriminate any-fallers vs. non-fallers. The fall-anchored Sway Area MCID translates continuous change into a clinical responder definition for trial enrichment.

Allele-Specific Antisense Oligonucleotide with Enhanced Specificity for Timothy Syndrome Type 1

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Background: Timothy syndrome type 1 (TS1) is a multi-system disorder caused by a heterozygous pathogenic variant in the

alternatively spliced exon 8A of CACNA1C, the $\alpha 1$ subunit of the L-type calcium channel CaV1.2. We have previously shown that the TS1 p.G406R variant caused delayed channel inactivation, resulting in excessive calcium influx in TS1 cortical neurons, activity-dependent changes in neuronal morphology, and GABAergic neuron defect. Interestingly, the TS1 variant also interferes with alternative splicing, amplifying its own expression and interfering with the developmental shift in exon utilization from exon 8A to exon 8, which is the major isoform in the adult brain. We designed an antisense oligonucleotide (ASO) to affect this splicing, but it was not TS1 allele specific. To preserve developmentally regulated expression, we sought to develop an ASO that is TS1 allele specific to treat this disorder.

Methods: We designed ASOs via gapmer, walking, and modification optimization and tested them via qPCR for CACNA1C exons 8 and 8A for splicing efficiency. We tested top ASO hits for efficacy in rescuing calcium-dependent immediate early gene expression via qPCR, as well as whole-cell patch clamp electrophysiology for barium current, capacitance, and voltage-dependent inactivation.

Results: Gapmers did not improve efficiency. We identified an ASO directly over the TS1 allele that specifically reduced TS1 exon 8A expression without reducing control exon 8A or exon 8 expression, termed ASO.B4. ASO.B4 rescued immediate early gene expression changes, specifically FOS and EGR expression. ASO.B4 also rescued electrophysiology measures including the known deficit in voltage-dependent inactivation.

Conclusions: These data provide evidence that the novel ASO.B4, optimized for its enhanced specificity, effectively rescues the pathological gating defects of L-type calcium channel kinetics in TS1, while leaving control CACNA1C exon 8A expression intact. We hypothesize this could reduce side effects from developmentally regulated isoform-specific expression.

Development of Antisense-Oligonucleotide Therapy for Histone 3.3-Related Bryant-Li-Bhoj Syndrome

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Background: Bryant-Li-Bhoj syndrome (BLBS) is a syndromic neurodevelopmental disorder with neurodegenerative features caused by heterozygous variants in either the H3F3A or H3F3B gene, both encoding the identical histone 3.3 (H3.3) protein. BLBS is caused by a range of missense variants throughout the H3F3A or H3F3B genes, and available clinical, cell and mouse data point to a gain-of-function mechanism. Although the shared disease mechanism across diverse variants remains incompletely understood, knock-down of the altered gene could ameliorate the disease phenotype. Antisense-oligonucleotide (ASO)-mediated knockdown of the affected gene or allele therefore could be therapeutic. Here, we investigate two alternate approaches that exploit the unique biology behind H3.3 production. In the first approach, we target both alleles of H3F3A or H3F3B. Given the inherent redundancy of H3.3 being encoded by two loci, this approach is expected to reduce the H3.3 protein no more than approximately 50%, allowing the targeting of only the gene affected by the pathogenic variant. The second approach targets the pathogenic variant in an allele-specific manner, thereby more selectively reducing the affected H3.3 protein, while preserving the three remaining alleles.

Methods: We have designed and screened 2'-methoxyethyl and phosphorothioate-backbone modified gapmer ASOs with both gene-specific and allele-specific designs. ASOs were transfected into 293T cells and H3F3A transcript level was determined by qPCR.

Results: Gene-specific ASO effectively reduced H3F3A transcript by over 90% in a cell culture system. Allele-specific ASO was designed for the recurrent T45I mutation in H3F3A, and while it achieved robust target knockdown, only a marginal selectivity towards the targeted allele was observed, highlighting the challenge of achieving high allele discrimination for single-nucleotide variants.

Conclusions: These data demonstrate ASOs as a potential therapeutic option for BLBS. ASOs were designed to target sequences with complete conservation between human and mouse, allowing for expedited evaluation of safety and efficacy in vivo, with particular attention to the tolerability of partial global H3.3 reduction.

Neuroinflammation and Neuroinfection

Allogenic Engineered T Cell Receptor Therapy for Progressive Multifocal Leukoencephalopathy

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Background: PML is a fatal disease of the central nervous system caused by JC polyomavirus (JCV). Survival is dependent on early diagnosis and ability to establish T cell immunity. Adoptive transfer of polyomavirus-specific T cells is possible; however, current delays in creating HLA-matched anti-viral T cells can be fatal. Off the shelf therapies are starting to become available, but the need to travel to receive them and the lack of consistent matching can result in mixed responses. We need an off the shelf option that can be rapidly deployed that takes advantage of recent engineering advancements to make effective allogenic T cells with a more consistent efficacy for a diverse array of patients.

Methods: PBMCs from healthy donors from a class I subtype were stimulated with peptide libraries tiled across the JCV VP1 protein. Multiple rounds of stimulation were performed to identify an epitope that induced the largest CD8+ T cell activation (i.e., INF-gamma, TNF-alpha, CD137, and CD69 expression). For these antigens, high-affinity antigen-specific CD8+ T cells were isolated using tetramers. Single-cell T cell receptor (TCR) sequencing identified the high-affinity, VP1-specific TCR-alpha/beta chain pairs. Candidate TCRs were both virally integrated via lentivirus and then precisely introduced with genome editing via CRISPR-Cas9 into the TRAC (T cell receptor alpha constant) locus into CD8 and CD4 donor T cells for further characterization. These T cells then underwent in vitro validation through antigen:MHC presentation on K562 cells and PML isolated non-cytolytic strains of JCV infected astrocytes. Further in vivo testing was completed by flank tumors of A375 tumor lines transduced to express antigen:MHCs of interest. The TCR also underwent further safety studies via alanine, X-scans, and autoreactivity scans. Further cell editing is being explored through both epigenetic edits and single gene knock-outs.

Results: A highly conserved region, as determined by a screen of >700 isolated viral copies of JCV isolated from PML patients, of the JCV VP1 protein (VP1 100-108) was identified as an antigen for HLA-A2*01 CD8+ T cells. Fourteen high avidity TCRs specific for VP1 100-108 were isolated, and ten demonstrated specific binding to VP1 100-108 HLA-A2 tetramers. The top two candidates were retrovirally edited into T cells and showed complete clearance of K627 cells expressing HLA-A2 compatible MHC with VP1100-108 at an ET of 2:1 after 48 hours of co-culture. Subsequently, we treated JCV infected astrocytes which showed CD137 activation of treatment T cells and 30-50% clearance of the infected astrocytes in increasing order of E:T after 72hr of co-culture. Based on this, we then used CRISPR-Cas9 editing to knock-in our TCR of choice and avoid any endogenous TCR effect. These T cells again showed efficacy in vitro. We then injected a A375 tumor line expressing HLA-A2 compatible MHC and VP1 100-108 into mice and showed complete tumor prevention and tumor growth reversal with our candidate T cell treatment. We have now explored this candidate TCR and found it to have a low risk of cross-reactivity via alanine and X-scan. In parallel, we have been optimizing editing techniques to allow for us to downregulate the expression of beta 2 microglobulin and CIITA, and upregulate CD47 for increased allogenic cell persistence. Other immunogenic regions of VP1 and candidate TCRs have been similarly identified for HLA-A3 allele.

Conclusions: We identified immunogenic epitopes from the JCV VP1 protein that bind HLA-A2 and A3 and isolated HLA restricted TCRs. For HLA-A2*01 we have demonstrated recognition and activation to the epitope of interest both in vitro and in vivo. We have further shown that targeting this epitope alone can clear around 40% of virally infected cells in vitro. While additional in vitro and in vivo validation are in progress, we believe these cells will offer a rapid, "off-the-shelf", allogeneic anti-JCV T cell therapy for PML patients who are unable to immune reconstitute.

CMV Viremia Is Associated with Poor Fungal Clearance from Cerebrospinal Fluid In Adult Patients With HIV-Associated Cryptococcal Meningitis

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Background: Cryptococcosis remains a leading cause of HIV-associated mortality globally. Cytomegalovirus (CMV) viremia is common in advanced HIV disease and is associated with excess mortality through unclear mechanisms. We assessed if CMV viremia is associated with slower rates of Cryptococcus clearance from cerebrospinal fluid (CSF) in persons being treated for HIV-associated cryptococcal meningitis.

Methods: We quantified baseline CMV DNA using PCR from cryopreserved plasma in adults with cryptococcal meningitis in Uganda from 2010 to 2024. The early fungicidal activity (EFA) was calculated as the negative slope of log₁₀ Cryptococcus colony-forming units (CFU)/mL CSF/day over 18 days. We compared baseline fungal burden, EFA, proportions achieving CSF sterility, and 18-week mortality by CMV status.

Results: Among 508 participants, 37% had detectable CMV viremia. Median EFA was lower in those with CMV viremia (0.31 vs 0.35 log₁₀CFU/mL/day; p=0.03), with a greater proportion having EFA <0.20 (30.5% vs 22.4%; p=0.04) and with fewer achieving CSF sterility by day 18 (36% vs 54%; p<0.001) than those without CMV viremia. Mortality at 18 weeks was higher in CMV viremic participants (43% vs 26%; p<0.001). Participants with both CMV viremia and EFA <0.20 CFU/mL/day had 2.4-fold increased hazard of death compared with those without CMV viremia and with EFA >0.20 CFU/mL/day (adjusted Hazard Ratio = 2.38 [95%CI, 1.51–3.75]). No interaction with antifungal treatment was observed.

Conclusions: CMV viremia is associated with impaired CSF fungal clearance and increased mortality in HIV-associated cryptococcal

meningitis. These findings support a potential indirect immunomodulatory role of CMV and warrant further investigation into the underlying immune response in HIV-CMV co-infection.

Neuromuscular Disease

Elucidating the Impact of Complex V Dimerization Defects on Mitochondrial Bioenergetics

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Background: ATP synthase (Complex V) dimerization plays a central role in shaping mitochondrial inner mitochondrial membrane (IMM) cristae, generating the high-curvature membrane architecture required for efficient oxidative phosphorylation (OxPhos). While this structural function is well established, it remains unclear whether the architectural role of ATP synthase dimers can be dissociated from their catalytic activity in ATP production and how cristae architecture contributes to the spatial organization of the proton motive force and supports adaptive responses to metabolic stress. Addressing this gap will have direct relevance for mitochondrial diseases, stress-induced energetic failure, and selective tissue vulnerability. Leigh syndrome (LS) causing pathogenic variants in ATP5MD, a small subunit required for dimer stability, selectively disrupt dimer formation while preserving monomeric ATPase activity, providing a unique system to dissect the relationship between IMM structure and bioenergetic function.

Methods: Live-cell dual-channel super-resolution fluorescence imaging was performed to simultaneously assess IMM architecture and membrane potential ($\Delta\Psi_m$) distribution at the sub-mitochondrial level. PKmito Deep Red was used as a structural marker of the IMM, and TMRM as a reporter of membrane potential. Imaging datasets were analyzed using a machine learning-based segmentation approach (Ilastik) to classify mitochondrial sub-compartments, including cristae membranes, inner boundary membrane, and matrix regions. These probability masks were integrated into a Python-based pipeline for automated, unbiased feature extraction, enabling quantification of structural features (e.g. membrane density, inter-cristae spacing) and functional parameters (compartment-specific TMRM intensity and inter-compartment $\Delta\Psi_m$ gradients). Bioenergetic function was assessed using Seahorse XF high-resolution respirometry under both basal and OxPhos-dependent (galactose) conditions to probe metabolic flexibility. Complex V organization and enzymatic activity were evaluated using native PAGE and spectrophotometric assays. Complementary lipidomic, proteomic, and transcriptomic analyses were performed to characterize alterations in membrane composition and respiratory chain organization.

Results: CRISPR-Cas9-generated ATP5MD knockout HAP1 cells demonstrated selective disruption of ATP synthase dimerization, confirmed by native PAGE, without significant impairment of monomeric ATPase catalytic activity, or reduction in isolated OXPHOS enzyme activity. This establishes a biochemical dissociation between the structural and enzymatic roles of Complex V. Structural analysis revealed marked alterations in IMM architecture, consistent with loss of the membrane-bending forces normally exerted by ATP synthase dimers and resulting in disorganized cristae morphology. Super-resolution imaging further demonstrated altered sub-mitochondrial $\Delta\Psi_m$ distribution, with reduced membrane potential across cristae membranes relative to inner boundary membrane and matrix compartments, indicating impaired compartmentalization of the proton motive force and disruption of bioenergetic microdomains. These preliminary findings support the hypothesis that ATP synthase dimerization is required for maintaining spatial organization of $\Delta\Psi_m$ within the IMM. Bioenergetic profiling revealed preserved basal respiration in glucose-rich conditions but failure to sustain maximal respiratory capacity under galactose-induced metabolic stress, mirroring the clinical phenotype of LS patients who decompensate under increased energetic demand. Lipidomic analysis demonstrated reduced levels of cardiolipin and associated biosynthetic intermediates, consistent with IMM remodeling and further supporting structural instability. Importantly, these findings were recapitulated and were more pronounced in ATP5MD mutant patient-derived fibroblasts, where cristae disorganization and $\Delta\Psi_m$ compartmentalization defects were more severe, likely reflecting differences in mitochondrial network architecture and cellular context. Notably, initial experiments using nonyl acridine orange (NAO) resulted in marked mitochondrial network fragmentation in patient fibroblasts, suggesting increased susceptibility of the IMM to structural perturbation and supporting the presence of intrinsic membrane vulnerability in dimerization-deficient states.

Conclusions: ATP synthase dimerization is a critical determinant of mitochondrial cristae architecture and the spatial organization of membrane potential within the IMM. Disruption of this structural function causes focal loss of $\Delta\Psi_m$ and reduced bioenergetic resilience under metabolic stress, despite preserved catalytic activity of ATP synthase monomers. These findings define a mechanistic link between membrane architecture and mitochondrial function, provide new insights into the pathophysiology of mitochondrial diseases and identify correction of cristae organization as a potential therapeutic strategy.

NEIMY-Associated KIF5A Mutation Impairs Trak-Mediated Mitochondrial Transport

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Abstract: KIF5A is a neuron-specific heterotetramer belonging to the Kinesin-1 family, a group of microtubule motor proteins that plays a large role in anterograde axonal transport and cytoskeletal regulation. KIF5A is one of three kinesin-1 isoforms, alongside the more ubiquitously expressed KIF5B and the motor neuron-enriched KIF5C. All three paralogs share a common structure: 2 kinesin heavy-chain domains that dimerize to form a stalk and 2 kinesin light-chains. Both of these structures allow kinesin-1 to bind to cellular cargo through the association of adaptor proteins, such as Trak2 for mitochondrial trafficking. Mutations in KIF5A have been linked to two distinct disorders: Amyotrophic Lateral Sclerosis (ALS), a progressive motor neuron disease, and Neonatal Intractable Myoclonus (NEIMY), a fatal form of infant epilepsy. Despite sharing similar frameshift mutations within a common region of KIF5A's C-terminal domain, these disorders exhibit distinct clinical phenotypes. ALS-associated KIF5A is well-studied, however, the distribution and intracellular impact of NEIMY-associated KIF5A remain unexplored. To investigate these distinct phenotypes, cells expressing WT, ALS, and NEIMY KIF5A mutations were used to assess the effect of these mutations on cargo-complex assembly. Our studies revealed that ALS and NEIMY KIF5A variants exhibit protein accumulation in the neurite tip as compared to WT, depleting KIF5A in the soma. In cells expressing KIF5A NEIMY, large aggregates form and disrupt axonal integrity by increasing axonal swelling and cytoskeletal damage. In cells with no kinesin heavy chain, there was a reduction in overlap between Trak2 and NEIMY KIF5A suggesting reduced efficiency of formation of cargo-complex. Finally, mitochondrial transport was shown to be dysregulated in a mutation-specific manner for both ALS and NEIMY KIF5A. These results taken together suggest different pathological mechanisms for ALS and NEIMY KIF5A that result in the disruption of autoinhibition, reduced formation of the cargo-complex, and differences in mitochondrial trafficking. These findings enhance our understanding of the pathophysiology of both diseases and shed light on the underlying biology of KIF5A itself.

Long-Read Amplicon Sequencing Method to Measure CTG Repeat Length and Mosaicism for Correlation to Disease Severity in Myotonic Dystrophy Type 1

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Abstract: Myotonic dystrophy type 1 (DM1) is a multisystemic disease caused by expansion of a CTG repeat in the DMPK gene. In DM1 the pathogenic repeats range in length from 100s to 1000s of triplets in blood cells of affected individuals. Using current methods, the length of the inherited repeat in the blood is broadly correlated with disease severity (Pearson's $R^2 = -0.44$; Age-of-onset x modal CTG length), but accurate measurement of individual repeat lengths has technical limitations, particularly with larger alleles. Identifying an accurate, cost-effective method for repeat length measurement is a critical step in ongoing efforts to identify novel genetic modifiers of DM1 or evaluate efficacy of repeat stabilizing therapeutics.

We set out to develop a method to measure CTG repeat length and mosaicism that is amenable to low sample input, economically feasible for large-scale analyses, and allows for sequence-level data. Using non-amplification methods as the standard, we compare (1) Southern blot, (2) small-pool PCR, (3) Cas9-mediated genomic DNA enrichment followed by PacBio long-read sequencing, (4) long-range PCR, and (4) PacBio sequencing of long-range PCR products. Fidelity of all methods are examined using a test-set of patient-derived blood gDNA samples with variable length and mosaicism, and gDNA mixes of known repeat lengths from patient-derived cell lines. We analyze concordance between the methods, examine the limitations of detection of longer or shorter alleles dependent on method, and quantification of mosaicism. We show that our long-range PCR can amplify highly expanded CTG repeat alleles and represent mosaicism. Incorporation of unique molecular identifiers to perform bioinformatic amplification correction is explored. Next, to assess the correlation of CTG repeat length and mosaicism with disease severity, we used PacBio sequencing of long-range PCR products correlated to age-of-onset and grip strength in participants of the END-DM1 natural history study.

Investigating Benefits of a Physical Therapist-Guided Exercise Program in Myotonic Dystrophy Type 2. NCT 06716931

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Abstract: Little is known about benefits of exercise in patients with myotonic muscular dystrophy type 2 (DM2). We designed a patient-friendly exercise program called PRIME (Proximal Resistance In-House Movement Exercises) that targets proximal muscles of lower extremities and core muscles as they are more frequently affected in DM2. We hypothesized that this exercise regimen improves functional capacity and muscle composition in DM2 patients when supervised by a physical therapist. Objectives: 1) To determine the benefits of PRIME by collecting patient- and clinician-reported outcome measures (PROMs and ClinROMs) that assess overall subject's health, functional and motor performance, fatigue, excessive daytime sleepiness, and breathing function, and 2) to determine exercise-induced muscle composition changes by using electrical impedance myography (EIM). Patients/Methods: To date, 9 DM2 patients completed the study. They were randomized (<https://ctrandomization.cancer.gov/tool/>) to one of the three possible cohorts (A, B, or C) in this two-period two-sequence cross-over study of 6 months duration. All patients performed two 45-

minute sessions of PRIME per week during this six-month study. PROMs and ClinROMs were collected at Day 1, 3-months (prior to cross-over) and 6-months (completion of the study). During the first 3 months, cohort A and B were partially or fully supervised by physical therapist during virtual exercise sessions; whereas cohort C patients performed exercise sessions on their own after learning exercises with a physical therapist at screening visit. During the last 3 months, cohort A and B patients performed exercise sessions on their own; whereas cohort C patients transitioned to virtual exercise sessions supervised by physical therapist. EIM was performed at screening visit, at 3 months, and at 6 months (completion of the study) for the following muscle groups bilaterally: deltoid, biceps, triceps, wrist flexors, wrist extensors, rectus femoris, vastus lateralis, vastus medialis, tibialis anterior and gastrocnemius medialis. Mixed-fixed and random-coefficient regression models were used as statistical analyses. Results: Patients' adherence to virtual exercise program was higher than patients' adherence to self-directed exercise program. High patients' satisfaction with exercise program was observed across all cohorts. Despite intraindividual improvement in pure motor outcome measures such as the six-minute walking test at completion of the study in most of the patients, fatigue daytime sleepiness score and Epworth sleepiness scale score (to screen for fatigue and daytime sleepiness) worsened in four and three patients at completion of study; respectively. EIM analyses are ongoing. Preliminary conclusions: Exercise improves pure motor outcome measures across all cohorts, however, exercise does not appear to improve fatigue and/or daytime sleepiness in this population.

Characterizing Energy Metabolism Among People With ALS And Pathogenic Gene Carriers

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Background: Hypermetabolism is common among person with Amyotrophic Lateral Sclerosis (PALS). We leveraged PALS with C9orf72 hexanucleotide repeat expansion (C9-ALS) and asymptomatic gene carriers (C9-carriers) to understand the relationships between energy metabolism alteration, neuronal degeneration, and disease progression.

Methods: C9-ALS, C9-carriers, disease controls with mitochondrial DNA defects (DC) and healthy controls (HC) were included. Demographics and blood samples were collected at enrollment. We measured total energy expenditure (TEE) with doubly labeled water (DLW), resting energy expenditure (REE) and respiratory exchange rate (RExR) with metabolic chamber, and fat mass (FM) with dual-x-ray-absorptiometry (DXA) or quantitative magnetic resonance (QMR). Fat-free mass (FFM) was calculated from body weight and FM. Plasma neurofilament light (NfL) and growth differentiation factor-15 (GDF-15) were measured with ELLA and ELISA, respectively. ANOVA and linear regressions (reference HC) were used for group comparisons, adjusted for age, sex and FFM as covariates.

Results: Total 55 participants were included, 8 C9-ALS, 16 C9-carriers, 10 DC, and 21 HC. C9-ALS was older than C9-carriers, DC and HC (65 vs 46 vs 46 vs 46, $p < 0.01$). Sex, height, weight, body mass index (BMI), FM and FFM were not significantly different between groups. REE was 203 kcal/day higher in C9-ALS compared to HC, adjusted for covariates, $p < 0.05$. TEE was 182 kcal/day higher in C9-carriers compared to HC adjusted for covariates, $p < 0.05$. RExR was lower in C9-ALS compared to HC (0.72 vs 0.78), $p < 0.01$. Compared to HC, NfL was higher in C9-ALS and GDF-15 was higher in both C9-ALS and DC, adjusted for age (all $p < 0.01$).

Discussion: High REE with low RExR, high blood NfL and GDF-15 levels are features of C9-ALS, signifying hypermetabolism with increased fatty acid oxidation, neuronal degeneration and cellular stress. The utility of these features as prognostic and predictive biomarkers in C9-ALS and gene-carriers is being evaluated in the ongoing study.

Neuro-Oncology

Targeting Processive Transcription for Myc-Driven Circuitry In Medulloblastoma

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Background: Medulloblastoma is the most common malignant brain tumor of childhood. The highest-risk tumors are driven by recurrent Myc amplifications (Myc-MB) and experience poorer outcomes despite intensive multimodal therapy. The Myc transcription factor defines core regulatory circuitry for these tumors and acts to broadly amplify downstream pro-survival transcriptional programs. Therapeutic targeting of Myc directly has proven elusive, but inhibiting transcriptional cofactors may present an indirect means of drugging the oncogenic transcriptional circuitry sustaining Myc-MB.

Methods: Independent CRISPR-Cas9 screens were pooled to identify conserved dependencies in Myc-MB. We performed chromatin conformation capture (Hi-C) from primary patient Myc-MB samples to map enhancer-promoter interactions. We then treated in vitro and xenograft models with CDK9/7 inhibitors to evaluate effect on Myc-driven programs and tumor growth.

Results: Eight CRISPR-Cas9 screens performed across three independent labs identify CDK9 as a conserved dependency in Myc-MB. Myc-MB cell lines are sensitive to CDK9 inhibition at low nanomolar concentrations, and these synergize with CDK7 inhibition. Inhibition of transcriptional CDKs disrupts enhancer-promoter activity within the Myc topologically associated domain, largely abrogating transcriptional output from the Myc promoter. This leads to a decrease in Myc binding genome-wide with a concordant downregulation of hallmark Myc-driven transcriptional programs. CDK9 inhibitors currently in clinical development lack CNS penetration, but novel agents such as ORI-CU-1 may overcome this limitation.

Conclusions: CDK9/7 catalytic activity represents a druggable vulnerability underpinning Myc-driven transcriptional programs. The development of CNS-penetrant CDK9/7 inhibitors may open new avenues for rational therapy in these high-risk medulloblastomas.

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Friday Networking Table Assignments

First Name	Last Name	INSTITUTION	Table 1	Table 2
Ahmed	Abdelhak	University of California, San Francisco	7	1
Nicolas	Abreu	New York University Grossman School of Medicine	6	6
Farwa	Ali	Mayo Clinic	8	5
Emanuele	Barca	Columbia University Medical Center	1	9
Alex	Bender	Massachusetts General Hospital	6	4
Jonathan	Brent	University of Pittsburgh	3	9
Matthew	Brier	Washington University in St. Louis	10	1
Sarah	Brooker	Northwestern University Feinberg School of Medicine	4	5
Samuel	Carrell	Virginia Commonwealth University	6	9
Sharon	Chiang	University of California, San Francisco	3	3
Jason	Chua	Johns Hopkins University	3	5
Prajwal	Ciryam	University of Maryland School of Medicine	5	7
Benjamin	Cox	University of Alabama at Birmingham	8	4
Vishnu	Cuddapah	Baylor College of Medicine / Texas Children's Hospital	1	1
Nathan	Dahl	University of Colorado School of Medicine	5	9
Melissa	Elafros	University of Michigan	9	8
Bolanle	Famakin	University of Wisconsin	6	10
Amena	Fine	Kennedy Krieger Institute/Johns Hopkins School of Medicine	9	11
D. Luke	Fischer	University of California, San Francisco	4	2
Taha	Gholipour	University of California San Diego	10	3
James	Giles	Yale University	7	10
Paloma	Gonzalez Perez	Mass General Brigham	9	9
Sasha	Gupta	University of California, San Francisco	5	8
Lauren	Hammer	University of Pennsylvania	10	6
William	Hayward	National Institute of Neurological Disorders and Stroke	7	2
Victoria	Ho	University of California, Los Angeles	2	3
Brandon	Holmes	University of California, San Francisco	3	2
Destiny	Hooper	Washington University School of Medicine	7	10
David	Isaacs	Vanderbilt Health	7	6
Sarah	Kaufman	University of California, San Francisco	4	2
Kevin	Keenan	University of California, Davis	10	10
Juliet	Knowles	Stanford University School of Medicine	4	3
Courtney	Lane-Donovan	University of California, San Francisco	5	11
Ikjae	Lee	Columbia University Irving Medical Center	8	9
Rebecca	Levy	Stanford University	2	11
Britta	Lindquist	University of California, San Francisco	5	10
Nijee	Luthra	University of California, San Francisco	8	6

Jessica	Magid-Bernstein	Yale University	10	7
Pamela	Marcott	University of California, San Francisco	4	5
Laura	McGarry	Children's Hospital of Philadelphia	2	4
Jillian	McKee	Children's Hospital of Philadelphia	6	4
Niccolo	Mencacci	Northwestern University	1	8
Andrew	Michalak	New York University Grossman School of Medicine	7	4
Jana	Mike	University of California, San Francisco	2	10
Julian	Motzkin	University of California, San Francisco	9	1
Hiroki	Nariai	University of California, Los Angeles	6	3
Tomoki	Nomakuchi	Children's Hospital of Philadelphia	1	11
Temitayo	Oyegbile-Chidi	University of California, Davis	8	4
Elizabeth	Pierpont	University of Minnesota	10	11
John	Pluvine	UCSF / Arc Institute	3	1
Jamie	Podell	University of Maryland School of Medicine	9	7
Altaf	Saadi	Massachusetts General Hospital, Harvard Medical School	9	8
Ethan	Sanford	UT Southwestern	6	7
Samuel	Shin	University of Pennsylvania	4	7
Caleb	Skipper	University of Minnesota	9	8
Alena	Stasenko	University of California, San Diego	8	4
Alexis	Steinberg	University of Pittsburgh	10	7
Andrew	Stern	Brigham and Women's Hospital	3	2
Sheng	Tang	Northwestern University	2	3
Sara	Trowbridge	Boston Children's Hospital	1	8
Jeff	Wagh	University of Texas Southwestern	8	6
Michael	Young	Massachusetts General Hospital	7	7
Julie	Ziobro	University of Michigan	2	4

Saturday – Breakout #1 Assignments

First Name	Last Name	Saturday Breakout #1	Room Name	Saturday Breakout #1 (Mentors)
Emanuele	Barca	1	Shorebreak 1	Barmada, Zeiger
Sarah	Brooker	1	Shorebreak 1	Barmada, Zeiger
Sharon	Chiang	1	Shorebreak 1	Barmada, Zeiger
D. Luke	Fischer	1	Shorebreak 1	Barmada, Zeiger
Pamela	Marcott	1	Shorebreak 1	Barmada, Zeiger
Tomoki	Nomakuchi	1	Shorebreak 1	Barmada, Zeiger
Matthew	Brier	2	Shorebreak 1	Boes, Blakeley
Melissa	Elafros	2	Shorebreak 1	Boes, Blakeley
Taha	Gholipour	2	Shorebreak 1	Boes, Blakeley
Ikjae	Lee	2	Shorebreak 1	Boes, Blakeley
Hiroki	Nariai	2	Shorebreak 1	Boes, Blakeley
Elizabeth	Pierpont	2	Shorebreak 1	Boes, Blakeley
Bolanle	Famakin	3	Shorebreak 2	Chen-Plotkin, Paulson
Brandon	Holmes	3	Shorebreak 2	Chen-Plotkin, Paulson
Courtney	Lane-Donovan	3	Shorebreak 2	Chen-Plotkin, Paulson
Niccolo	Mencacci	3	Shorebreak 2	Chen-Plotkin, Paulson
Jana	Mike	3	Shorebreak 2	Chen-Plotkin, Paulson
Andrew	Stern	3	Shorebreak 2	Chen-Plotkin, Paulson
Ahmed	Abdelhak	4	Shorebreak 2	Chu, Wilson
Nicolas	Abreu	4	Shorebreak 2	Chu, Wilson
Kevin	Keenan	4	Shorebreak 2	Chu, Wilson
Jessica	Magid-Bernstein	4	Shorebreak 2	Chu, Wilson
Ethan	Sanford	4	Shorebreak 2	Chu, Wilson
Alexis	Steinberg	4	Shorebreak 2	Chu, Wilson
Farwa	Ali	5	Driftwood 1	Cross, Miller
Paloma	Gonzalez Perez	5	Driftwood 1	Cross, Miller
David	Isaacs	5	Driftwood 1	Cross, Miller
Nijee	Luthra	5	Driftwood 1	Cross, Miller
Temitayo	Oyegbile-Chidi	5	Driftwood 1	Cross, Miller
Altaf	Saadi	5	Driftwood 1	Cross, Miller
Amena	Fine	6	Driftwood 1	Golshani, Paredes
Rebecca	Levy	6	Driftwood 1	Golshani, Paredes
Samuel	Shin	6	Driftwood 1	Golshani, Paredes
Laura	McGarry	6	Driftwood 1	Golshani, Paredes
Andrew	Michalak	6	Driftwood 1	Golshani, Paredes
Sheng	Tang	6	Driftwood 1	Golshani, Paredes
Jonathan	Brent	7	Driftwood 2	Kao, Khoshkoo
Jason	Chua	7	Driftwood 2	Kao, Khoshkoo
Nathan	Dahl	7	Driftwood 2	Kao, Khoshkoo
Julie	Ziobro	7	Driftwood 2	Kao, Khoshkoo
Prajwal	Ciryam	8	Driftwood 2	Koshy, Coufal
Sasha	Gupta	8	Driftwood 2	Koshy, Coufal
Sarah	Kaufman	8	Driftwood 2	Koshy, Coufal

Destiny	Hooper	8	Driftwood 2	Koshy, Coufal
John	Pluinage	8	Driftwood 2	Koshy, Coufal
Caleb	Skipper	8	Driftwood 2	Koshy, Coufal
Alex	Bender	9	Driftwood 1	Liu, Goldberg
Benjamin	Cox	9	Driftwood 1	Liu, Goldberg
William	Hayward	9	Driftwood 1	Liu, Goldberg
Britta	Lindquist	9	Driftwood 1	Liu, Goldberg
Julian	Motzkin	9	Driftwood 1	Liu, Goldberg
Michael	Young	9	Driftwood 1	Liu, Goldberg
Vishnu	Cuddapah	10	Driftwood 2	Nelson, Mattis
Victoria	Ho	10	Driftwood 2	Nelson, Mattis
Juliet	Knowles	10	Driftwood 2	Nelson, Mattis
Sara	Trowbridge	10	Driftwood 2	Nelson, Mattis
Jeff	Waugh	10	Driftwood 2	Nelson, Mattis
Samuel	Carrell	11	Shorebreak 2	Lesli-Mazwi, Guilliams
James	Giles	11	Shorebreak 2	Lesli-Mazwi, Guilliams
Lauren	Hammer	11	Shorebreak 2	Lesli-Mazwi, Guilliams
Jillian	McKee	11	Shorebreak 2	Lesli-Mazwi, Guilliams
Jamie	Podell	11	Shorebreak 2	Lesli-Mazwi, Guilliams
Alena	Stasenko	11	Shorebreak 2	Lesli-Mazwi, Guilliams

Saturday – Breakout #2 Assignments

First Name	Last Name	Saturday Breakout #2	Room Name	Saturday Breakout #2 (Mentors)
Jason	Chua	1	Driftwood 1	Barmada, Lee
Vishnu	Cuddapah	1	Driftwood 1	Barmada, Lee
Victoria	Ho	1	Driftwood 1	Barmada, Lee
Hiroki	Nariai	1	Driftwood 1	Barmada, Lee
Jana	Mike	1	Driftwood 1	Barmada, Lee
Andrew	Stern	1	Driftwood 1	Barmada, Lee
Alex	Bender	2	Driftwood 1	Boes, Khoshkoo
D. Luke	Fischer	2	Driftwood 1	Boes, Khoshkoo
Jillian	McKee	2	Driftwood 1	Boes, Khoshkoo
Sarah	Kaufamn	2	Driftwood 1	Boes, Khoshkoo
Sheng	Tang	2	Driftwood 1	Boes, Khoshkoo
Sarah	Brooker	3	Driftwood 2	Brooks-Kayal, Miller
Britta	Lindquist	3	Driftwood 2	Brooks-Kayal, Miller
Pamela	Marcott	3	Driftwood 2	Brooks-Kayal, Miller
Laura	McGarry	3	Driftwood 2	Brooks-Kayal, Miller
Tomoki	Nomakuchi	3	Driftwood 2	Brooks-Kayal, Miller
Jonathan	Brent	4	Driftwood 2	Coufal, Golshani
Nathan	Dahl	4	Driftwood 2	Coufal, Golshani
Bolanle	Famakin	4	Driftwood 2	Coufal, Golshani
Brandon	Holmes	4	Driftwood 2	Coufal, Golshani
Julie	Ziobro	4	Driftwood 2	Coufal, Golshani
Sara	Trowbridge	4	Driftwood 2	Coufal, Golshani
Ethan	Sanford	5	Shorebreak 1	Cross, Paredes
ALENA	STASENKO	5	Shorebreak 1	Cross, Paredes
Alexis	Steinberg	5	Shorebreak 1	Cross, Paredes
Michael	Young	5	Shorebreak 1	Cross, Paredes
Sharon	Chiang	6	Shorebreak 1	Goldberg, Koshy
William	Hayward	6	Shorebreak 1	Goldberg, Koshy
Rebecca	Levy	6	Shorebreak 1	Goldberg, Koshy
Niccolo	Mencacci	6	Shorebreak 1	Goldberg, Koshy
John	Pluvineage	6	Shorebreak 1	Goldberg, Koshy
Ahmed	Abdelhak	7	Shorebreak 1	Guilliams, Liu
Benjamin	Cox	7	Shorebreak 2	Guilliams, Liu
Jessica	Magid-Bernstein	7	Shorebreak 2	Guilliams, Liu
Julian	Motzkin	7	Shorebreak 2	Guilliams, Liu
Jamie	Podell	7	Shorebreak 2	Guilliams, Liu
Juliet	Knowles	8	Shorebreak 2	Lesli-Maswi, Blakeley
Matthew	Brier	8	Shorebreak 2	Lesli-Maswi, Blakeley
Temitayo	Oyegbile-Chidi	8	Shorebreak 2	Lesli-Maswi, Blakeley
David	Isaacs	8	Shorebreak 2	Lesli-Maswi, Blakeley
Nijee	Luthra	8	Shorebreak 2	Lesli-Maswi, Blakeley
Altaf	Saadi	8	Shorebreak 2	Lesli-Maswi, Blakeley

Prajwal	Ciryam	9	Shorebreak 2	Mattis, Zeiger
Sasha	Gupta	9	Shorebreak 2	Mattis, Zeiger
Courtney	Lane-Donovan	9	Shorebreak 2	Mattis, Zeiger
Emanuele	Barca	9	Shorebreak 2	Mattis, Zeiger
Samuel	Shin	9	Shorebreak 2	Mattis, Zeiger
Samuel	Carrell	10	Shorebreak 1	Nelson, Chen-Plotkin
James	Giles	10	Shorebreak 1	Nelson, Chen-Plotkin
Destiny	Hooper	10	Shorebreak 1	Nelson, Chen-Plotkin
Caleb	Skipper	10	Shorebreak 1	Nelson, Chen-Plotkin
Andrew	Michalak	10	Shorebreak 1	Nelson, Chen-Plotkin
Nicolas	Abreu	11	Driftwood 1	Paulson, Kao
Amena	Fine	11	Driftwood 1	Paulson, Kao
Taha	Gholipour	11	Driftwood 1	Paulson, Kao
Lauren	Hammer	11	Driftwood 1	Paulson, Kao
Kevin	Keenan	11	Driftwood 1	Poduri, Kao
Farwa	Ali	12	Driftwood 2	Wilson, Chu
Melissa	Elafros	12	Driftwood 2	Wilson, Chu
Paloma	Gonzalez Perez	12	Driftwood 2	Wilson, Chu
Ikjae	Lee	12	Driftwood 2	Wilson, Chu
Elizabeth	Pierpont	12	Driftwood 2	Wilson, Chu
Jeff	Waugh	12	Driftwood 2	Wilson, Chu